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EMBRYOLOGY AND DEVELOPMENT

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The basic developmental pattern of this chapter was laid down by the late Peter Williams; it has provided a sound foundation. The dramatic advances of molecular biology since the last edition have produced vast amounts of data on developing systems, synthesis of which has provided some insight into common embryonic processes occurring in all systems of the body. An account of the diverse development of each system has only been possible because of the specialist advice and help from many contributors, each experts in their fields. Significant portions of the text have been revised by Mark Ferguson, Sarah Guthrie, Frank Billett, Sue Kimber and John Aplin; and contributions have been gratefully received from Aidan Breathnach, Nigel Brown, Duncan Davidson, John Carroll, Nicole Le Douarin, Bob Edwards, Anne Ferguson-Smith, Tom Fleming, Peter Holland, Martin Johnson, Anthony Lander, Barry Mitchell, Isabella Moore, Antoon Moorman, Gonzalo Moscoso, Charles Roedeck, Paul Sharpe, Richard Sharpe, Cheryl Tickle and Michael Whitaker. Illustrations have been provided by Kevin Marks, Peter Lamb, Peter Jack, Jenny Halstead, Mark Hay and Andrew Bezeal and Denise Smith.

This chapter has evolved in its revision reflecting the changing and improving techniques now used for examining embryos. It has also grown by interstitial and accretionary growth, to encompass the increase in the depth and extent of our understanding of developmental processes.

Although the chapter has been written to provide the most up to date account of the subject as we go to press, I hope it is also accessible to a wide range of students who hopefully will become inspired by the subject and contribute to it in the future.

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Introduction

The life cycle passes through phases, from gametes to zygote, embryo to fetus and finally juvenile to adult. Revolution of the cycle is ensured by the repetition of this sequence. In humans, gamete production occurs in the female during embryonic life and is completed prior to birth; in males gamete cell lines are stored during the embryonic and juvenile stage ready for further replication in adulthood. In terms of the propagation of individuals, the accurate copying of DNA during gametogenesis, the inclusion of variation in the code during meiosis and the successful production of a viable juvenile, which is able to pass on its DNA, are the major features of the cycle. The mechanisms which control phases of the cycle are contained within the genome and expression of genes and their products, in particular sequences, result in the morphological changes seen during embryo formation. Study of the interactions of molecules responsible for such morphological changes forms the basis of developmental biology. The embryology and development section is initially concerned with those basic features of development which are common to all multicellular (metazoan) animals, more especially chordate craniates, particularly as viewed in the light of their evolutionary history. The experimental work, genetic analysis and the concepts of the later 20th-century developmental biology are outlined. Following a description of the earliest assumption of embryonic morphology, the development of the human embryo is considered in detail in relation to the development of individual systems; in each case descriptions of embryonic morphology are

supported by experimental evidence of the underlying molecular mechanisms. The consequences of error in developing systems are examined.

The Comparative Principle

There are two important, indeed fundamental, approaches to anatomical study which lead to a significantly deeper understanding of the structure of the human body not only at the gross and histological levels but also, increasingly, at the molecular level; these concern the **comparative** and **developmental** aspects, particularly through the study of craniate/vertebrate chordates and their subgroups the anamniotes and amniotes. The importance of both aspects is deeply rooted in the history of the discipline of anatomy.

The science of *comparative anatomy* was founded during the latter half of the 18th century and became established during the first half of the 19th century. A notable British contribution was made by the great physiologist and surgeon John Hunter (1728–93) whose vast collection of some 14 000 specimens, many of them fossils and skeletal structures, was subsequently sorted and catalogued by Richard Owen (1804–92) to form the basis of the Hunterian Museum of the Royal College of Surgeons. Owen was influenced greatly by the ideas of 'transcendental anatomy' and 'idealistic morphology' emanating especially from Germany (Goethe, 1739–1842) and France (Geoffroy Saint-Hilaire, 1772–1844) which fostered the notion that organisms were related through common anatomical design. Of particular interest is Owen's distinction between homologous (same basic design) and analogous (same function) structures; also noteworthy was his concept of a *vertebrate archetype*, which laid particular emphasis on the *segmental organization* of the skeleton. The theme of comparative anatomy, that there is a basic pattern to all vertebrate structure, was enhanced by the gradual realization that this pattern emerged during *embryonic development* in a relatively simple form. The concept of the *germ layers*, implying the invariant origin of tissues, initially formulated by von Baer (1828^[1]), together with his emphasis that during development generalized structure precedes specific and specialized structure, cemented the link between the developmental and comparative aspects.

The publication of Darwin's *The Origin of Species by Natural Selection* (1859^[2]) provided the third, and critically important, element towards an enlightened approach to the study of human anatomy in general and to embryology in particular. Although the famous dictum of Haeckel (1874^[3]) that ontogeny (development) recapitulates phylogeny (evolution) encapsulates a view that is still regarded as controversial, nevertheless, it provides a useful guideline for the appreciation of the emergent pattern of the vertebrate embryo as it passes through phases shared by a common ancestor.

Concepts and Terminology

Almost all of the terms which were employed to describe the development of chordates came into use during the second half of the 19th century. The acceptance of a generally understood,


and internationally acceptable, terminology was essential to the emergence of embryology as a true **numerate, experimental science** in its own right. However, during the closing decades of the century important **concepts** became **embodied** in terms which implied support for the comparative principle based on the dominance of the germ layer theory and on the rarely questioned view that the embryological development of animals **recapitulated** important facets of their evolution.



An examination of the origin and meaning of the terms currently used to describe embryonic development reveals a list still dominated by 19th-century terminology. The familiar terms *morula*, *blastula* and *gastrula* derive from Haeckel's (1874^[1]) view that early stages of development correspond to a sequence whereby, during evolution, a simple multicellular mass of cells initially gave rise to a hollow sphere; subsequent invagination of part of the outer surface of the sphere produced a two-layered structure, designated by Haeckel as the *gastreae* stage of evolution, equivalent to the relatively simple gastrula stage of many marine organisms, for instance that of the sea urchin (p. 96^[2]). This initial invagination of embryonic cells was thus termed *gastrulation*. Although the progression envisaged by Haeckel was hypothetical, it is important to understand that it was driven by the evolutionary imperative and derived much of its plausibility from observations of embryos.

The notion that gastrulation is the most important phase of development is historically related to its association with the germ layer theory, particularly in relation to chordates. Basically this theory, originally formulated by von Baer (1828^[3]) and later refined by the brothers Hertwig (1879–83), states that the structure of all **animals** above the level of coelenterates is derived from **three embryonic layers**, namely an outer *ectoderm*, an inner *endoderm* and an intervening *mesoderm*, each layer generating specific tissue components corresponding to the triploblastic design of the postembryonic form. In vertebrates, for instance, it was envisaged that the ectoderm gave rise to the epidermis and the major part of the nervous system; the endoderm generated the epithelial lining of the gut and contributed to derived glandular structures as well as to the lining of the lungs; and the mesoderm formed the vascular system and much of the musculoskeletal structure.





Although the terms ectoderm, mesoderm and endoderm quickly became established and were, from the outset, commonly used to describe the germ layers, the terms *epiblast* or *ectoblast* (outer embryonic layer), *mesoblast* (middle embryonic layer) and *hypoblast* or *endoblast* (inner embryonic layer) were for many years recognized alternatives (Lankester 1877^[4]; Balfour 1888^[5]). Sedwick (1902) in fact suggested that the term mesoblast was **the** appropriate term for the middle layer of amniotes. Certainly in some ways the '*blast*' terminology is preferable; it retains the embryological connotation implicit in many other terms (e.g. *blastoderm*, *blastocyst*, *blastopore*) and clearly relates to the derived term *triploblastic*.


Currently the terms epiblast and hypoblast are used to describe the upper and lower cell layers of the *amniote* blastoderm respectively. Recently the term mesoblast has been reinstated both to describe the middle layer of amniote embryos and to designate mesenchymal cells produced from the primitive streak (see also p. 124^[6]). In amniotes both *embryonic* and *extraembryonic*


tissues (*membranes*) develop. The extraembryonic tissues are derived from both the epiblast and hypoblast, (see p. 98) , whereas the embryonic tissues are derived from the epiblast alone. This critical division of cell lineages into embryonic and extraembryonic distinguishes the *amniota* from the *anamniota* where all the cells derived from the zygote are embryonic (see below).

Despite the subsequent modification of the germ layer theory and the rejection of the idea of the *invariant* fate of cells derived from particular layers, the fundamental insight that the theory provided towards an enlightened understanding of the relation between embryonic development, histology and gross anatomical structure cannot be underestimated. The 19th-century embryologists recognized a close association between the process of gastrulation and the formation of the middle embryonic layer. However, Balfour (1888) and others frequently made a distinction between the two processes, because in some embryos other methods of forming the middle layer were envisaged. Largely as a result of experimental studies (see below), the modern view of gastrulation is that of a *dynamic process* which shifts prospective cell populations at, or associated with, the surface of the early embryo to its interior, resulting in the *tripartite division* of material from which the basic structure of the embryo, and thus that of the adult, is derived. Although vertebrate eggs may differ greatly in size and thus undergo strikingly different cleavage patterns after fertilization, they achieve through gastrulation a remarkably similar result (see p. 100) . It is in this context that gastrulation, as the essential **prerequisite** for the determination of triploblastic structure, may indeed be regarded as the single most important event in, and a vital concept of, embryonic development.


The use of the germ layers as a focus to describe early development has proved problematical in recent years due to the inflexibility of the terminology and its non-specific usage by the novice and the expert. The appeal of three layers from which all structures derive is enduring. However, it was known at the time the theory was gaining popularity that its focus on three layers was simplistic and misleading. The acceptance of other ways of deriving embryonic cells, apart from at gastrulation, for example by ingression of neural crest cells (see below), was slow. Changes in embryological terminology pertinent to the germ layers were not incorporated into many embryological texts, skewing the perceived value of some cell populations compared to others. The consequence has been that the words used to describe the middle layer of the embryo—mesoderm, mesoblast, and mesenchyme (see below)—are frequently used synonymously and without regard to changes in cell-cell contact and cell status; writers have ignored particularly the *transition from epithelium to free cells and vice versa*, such events being of significant importance during development. This vague use of terminology has inhibited the precise description of the origin and interaction of embryonic tissues.

It is apparent that the simple invagination of a sheet of epithelial cells by the deformation of a ball of cells will involve different cellular mechanisms from the separation of individual cells from an epithelium to a free state (as occurs at the primitive streak, see p. 142) . Cells which were seen free in groups within an embryo, with copious extracellular matrix, were termed *mesenchyme* by Hertwig (1881) and were described by Lankester (1877) as *mesoblast*—'cells which wandered through the matrix between the inner and outer layers of the embryo'. Hay (1968) suggested the terms *primary mesenchyme* for those cells formed by ingression at the primitive streak, and *secondary mesenchyme* for those formed by ingression at other sites, for

example the neural crest (see p. 147). She noted that the mesenchymal cells formed from the streak usually revert to an epithelial status when they reach their destination. Thus the populations of mesenchymal cells within a triploblastic embryo include, inter alia: mesenchymal cells which may differentiate along a connective tissue lineage, migrating epithelial cells which display a mesenchymal phenotype whilst migrating, and stem cells which may retain the ability to differentiate along different lines according to the local environmental influences. Recombinant experiments now allow mesenchyme populations to be exchanged to see if their site of origin is important in determining their final fate. Thus the specificity with which cells are termed assists understanding of their possible developmental fates.

A **clarification** of the terminology associated with the middle layer seems to be appropriate for the following reasons. Unlike the terms ectoderm and endoderm, which fittingly describe the outer and inner epithelial layers of the embryo, the term mesoderm, as currently used in *amniote development*, applies to an embryonic tissue which can be either epithelial or mesenchymal. The recognition of the importance of epithelial/mesenchymal interactions during development necessitates that the cellular arrangements within the embryo are described specifically. For this reason the term mesoblast is subsequently used in this section to denote a temporary, embryonic cell lineage, which will later generate **either** an epithelial **or** a free cell arrangement. The free cell arrangement will be termed *mesenchyme*; it will be designated according to its position or fate within the embryo. The *epithelial arrangement* will be specified with reference to its structure, prospective fate and location. In view of its dynamic nature mesenchyme should no longer be regarded as 'embryonic connective tissue'. The reader should note that the traditional term—mesoderm—will be retained in the brief account of prospective fate maps of blastulae and gastrulae in *anamniota*, and occasionally in other sections of this volume (for discussion see Collins & Billett 1995.

Reproduction

Reproduction, the ability of an organism to reproduce a more or less exact replica of itself, is a fundamental feature of living material; it is a property which at the onset of the origin of life (see p. 3) would have established the boundary between systems with the attributes of life and their complex, non-living, biochemical precursors. The advent of cellularization, which conserved complexity, ensured its survival by replication but allowed the possibility of genetic variation (through mutational change) and laid the foundation for a primitive reproductive capacity capable of further evolution, initially by **asexual** means but ultimately by a **sexual** method.

Gametogenesis

Although asexual methods of reproduction are common among relatively simple animals, and are sometimes incorporated into the life cycle of more advanced forms (insects), sexual reproduction is found throughout the animal kingdom. A multicellular organism which reproduces sexually consists mainly of general body (*somatic*) cells which, although exhibiting extreme ranges of variation in size, shape and specific functional as well as morphological

characteristics, all possess certain common features of their genetic apparatus. This contrasts with the highly distinctive differentiation process and genetic changes which occur within the *gonads* and result in the formation of mature *germ* cells or *gametes*. General somatic cells usually possess a full or *diploid number* of chromosomes, a half of which was originally derived from each parent. However, the *activity* and *expression* of the many different gene loci on the chromosomes varies in different cell types in adult tissues, and during development as differentiation occurs. The somatic cells which are capable of dividing do so by the process of mitosis (p. 57📖). Each chromosome, having first made a faithful replica of itself and thus of its genes, divides longitudinally and each resultant cell is again equipped with the diploid number of chromosomes which are replicas of those in the parent cell. During *gametogenesis*, however, a complex series of changes termed *meiosis* are set in train (p. 60📖). Essentially, during this, the original chromosome number is reduced to a half, the *haploid number*, in which a *recombination* of the genetic material has occurred. (Details are considered on p. 56📖.) The male gonad or *testis* produces many small motile gametes or *spermatozoa* in which the cytoplasmic machinery is much reduced; each consists of a nucleus bearing the genetic apparatus, the chromosomes, with a closely applied acrosome derived from a Golgi apparatus and succeeded by a long flagellum which is partly ensheathed by an energy-transforming mitochondrial sheath (p. 125📖). In contrast the female gonad or *ovary* produces fewer, larger, non-motile *ova* (eggs), their cytoplasm containing a variable quantity of food reserves (*yolk* or *deutoplasm*). The fusion of the gametes forms a *zygote* and restores the characteristic diploid chromosome number of the species. Most importantly, however, as the chromosomes are derived from parents which are genetically dissimilar, the sexual process fosters genetic variation, in contrast to the asexual method where it is limited to mutational change.

Influence of Egg Size and Structure

The pattern of early development is greatly influenced by the size of the egg destined to become an embryo; this particularly affects the stages of cleavage and gastrulation (see below) (3.1👁️). Depending almost exclusively on their yolk (nutritional reserve) content, eggs vary enormously in size, ranging from c.100 μm in diameter (e.g. for sea urchins and mammals) to over 1 cm (most birds and many reptiles); if volumes are compared, related to the power of 3, the difference is obviously even more striking. This difference in size is concerned with such factors as the need to disperse the progeny as **quickly** as possible, through the agency of a larval form (e.g. teleosts and sea urchins), or the need to **sustain** embryonic development for as long as possible, in the virtually closed environment of a *cleidoic egg* (e.g. birds). In the first case large numbers of small eggs are produced by the female and fertilization is **external**, in the second only a few large eggs are produced, for which **internal** fertilization is a prerequisite. The situation among present-day reptiles, birds and mammals (collectively referred to as *amniotes*) is particularly relevant to that which now exists in man. Both birds and mammals evolved from reptilian precursors (see p. 5📖) who by their ability to lay eggs which could develop on land achieved complete independence of the general aqueous environment to which their amphibian ancestors were restricted. (Better eggs rather than stronger legs were essential for the conquest of land.) The problems posed by the closed environment of the egg, relating to the provision of a local aqueous milieu, respiration, efficient utilization of the stored yolk and the enforced storage of excretory products were solved by the evolution of *extraembryonic membranes* (see below).

The reptilian precursors of the eutherian mammals at first retained their yolk-laden eggs and then utilized the existing membrane structures to make contact with the female parent, thus initiating the evolution of the varieties of *placentae* and complete dependence of embryonic development on the mother. Egg retention virtually eliminated the need for yolk with a consequent *secondary reduction* in the size of the egg to that now seen in mammals. This trend can be detected among a few present-day reptile groups, for example skinks. Thus although the eggs of mammals are **similar** in **size** to those of many marine invertebrates their evolutionary history has produced a **fundamental difference** in structure; this **must** be borne in mind when their development is considered.


Apart from size, embryonic development is determined, in a fundamental way, by the *intrinsic molecular structure* of the egg. In anamniotes, this structure is generated during oögenesis and subsequently modified by oöcyte maturation which transforms the diploid oöcyte into the haploid egg. This process confers a distinct *radial symmetry*, centred around the animal–vegetal axis, in many eggs. (A cephalocaudal axis in the case of insects.) The subsequent formation of a dorsoventral axis, usually by fertilization, establishes *bilateral symmetry*. The time of appearance, degree and reversibility of the appearance of this bilateral symmetry varies greatly between animal groups; in some cases the bilateral symmetry appears to be directly related to the point of sperm entry, but frequently it is not. The comparative and experimental evidence for the existence of this *inherent* symmetry, its relative fixity and lability, and especially the role of the egg cortex, was established many years ago. With the recent discovery of genes which determine polarity it remains an important focus of current research.

Egg structure provides the *heterogeneous cytoplasmic environment* which interacts with the *genetic programme* emanating from the zygote nucleus at the beginning of development. The subsequent progressive interaction and mutual modification of *cytoplasmic* and *nuclear components* was the basis of Morgan's (1934^[4]) explanation of the generation of diverse cell types during embryonic development.


The experimental analysis of the *causal mechanisms* of development (see p. 103^[4]) has revealed that *zygotes* exhibit some degree of segregation of cytoplasmic elements (determinants). However, the degree, precision and lability of this segregation varies greatly among animal groups. In some invertebrate eggs, for instance those of platyhelminthes, molluscs and annelids, a highly ordered structure is associated with a precise pattern of *spiral cleavage*, although in others, such as ascidians, it is not. With this type of egg structure the fate of the initial cleavage cells is fixed, and a cell separated at this stage results in the development of a partial embryo; cleavage of this kind is referred to as *determinate*, and proceeds to *mosaic development*. In contrast eggs possessing a more labile cytoplasmic structure are referred to as *indeterminate*; they undergo *regulative development*. Here each of the first few cells of the embryo has the capacity to form a complete although (obviously) smaller embryo (see p. 102^[4]). However, it is important to note that the implied distinction between the two types of egg structure is only one of degree; even the most mosaic eggs retain some regulative capacity (conferred by polar lobe material) and similarly most regulative eggs possess some degree of mosaicism.

Fertilization

Fertilization, the fusion of egg and sperm, achieves many purposes: it restores the chromosomal complement of the species; it determines the chromosomal sex of the new individual possessing either XX or XY sex chromosomes; it fosters genetic variation (in contrast to the asexual method where the variation is limited to mutational change) because the chromosomes are derived from genetically dissimilar parents; and it also activates the developmental potential of the egg itself. The presence of an X or Y chromosome has far-reaching consequences on all body systems, especially in terms of embryonic growth rate and final size, on the later proportions of muscle and fat in the body and also on some brain and spinal cord nuclei. *Activation*, which can also be achieved artificially or parthenogenetically, initiates the first stage of the reproductive process, the formation of the embryo. The reproductive cycle is completed by the production of sexually mature individuals capable of forming gametes, ensuring the continuation of the species-specific replicative process.


For all vertebrates and most invertebrates the process of reproduction, leading to maturity, is a long and complicated one. It is not so with simpler organisms which may merely divide after reaching the requisite level of complexity; here the time between successive generations (the reproductive period) is short and favours a rapid increase in number (as in bacteria and protozoa). The reproduction of an organism can only be said to be complete when it is itself capable of, or participating in, the reproductive process. In metazoan animals three phases can be recognized. The first of these is *embryogenesis* (which is considered in the following section); it is followed by a period dominated by *growth* (see p. 365 ) and finally by the phase leading to *sexual maturity*.

Embryonic Development

Fertilized, activated eggs containing both male and female pronuclei are termed *zygotes*. All zygotes undergo, initially, similar developmental processes; these are *cleavage* and *gastrulation*. Those zygotes which develop in cleidoic eggs also produce *extraembryonic membranes* which enable the nutritive yolk to be supplied to the embryo, establish a respiratory exchange, allow waste products to be removed and permit an aqueous environment to develop around the embryo itself. Similar membranes develop in mammalian embryos but they are especially concerned with establishing a connection between the embryonic tissues and the maternal circulation, i.e. a *placenta*. Development to this end is termed *implantation*. Thus in mammals generally, and for man in particular, stages of embryonic development are also subdivided. A distinction is often made between development occurring before the zygote has established a firm connection with the mother; this is sometimes referred to as *preimplantation development* (a stage when manipulation of the zygote can occur; see p. 132 ) and subsequent development which is termed *postimplantation*. The earliest development of all embryos (postimplantation in mammals) is termed *primary embryogenesis*, a stage when cell populations are formed and massive cell migrations occur. Later, the development of organs and systems is referred to as *organogenesis*. When mammalian embryos reach a certain size, growth rather than

morphogenesis occurs. The embryo is referred to as a *fetus*; this occurs at 56–57 postovulatory days in humans when the onset of bone marrow formation in the humerus can be seen (Streeter 1949⁴); at this stage more than 90% of the named structures of the adult body have appeared. The term *conceptus* defines the embryo (or fetus) plus its associated extraembryonic (or fetal) membranes.

Cleavage

Cleavage is the process by which the first mitotic divisions of the zygote produce the founder cells of the embryo, and, in amniotes, also the cells which give rise to the extraembryonic membranes. The process, by dividing the large amount of egg cytoplasm between many smaller cells, restores the nuclear/cytoplasm ratio of the cells; little growth occurs during this time. The first cells formed by cleavage are called *blastomeres*. The pattern of cleavage differs between (and frequently within) the Classes: it depends upon the amount of yolk in the zygote and the factors within the cytoplasm which influence the timing of mitosis and the angle of the mitotic spindle. Divisions can occur through the *animal* and *vegetal poles* of the zygote—*meridionally*, or between the poles—*equatorially*; the divisions can also produce *equal* or *unequal*-sized daughter cells. In most species apart from mammals the rate of cell division and the position of the blastomeres is patterned by *maternal factors* in the cytoplasm; the genome of the zygote does not appear to function in these early stages. The differences in cleavage pattern will be briefly described (3.1 )

In the **sea urchin** which has small (molecithal) eggs and thus small zygotes, the first two cleavage planes pass meridionally and at right angles to each other; the third cleavage is equatorial. Subsequent cleavages, however, are different for those cells at the animal pole and those at the vegetal. Ultimately a hollow *blastula* (a ball of cells with a central cavity or *blastocoele*) will be formed. This cleavage pattern is described as *radial holoblastic* (complete) cleavage, the same as in *Amphioxus*.

The **amphibian** zygote is mesolecithal: it contains much more yolk concentrated in the vegetal pole, which prevents initial symmetric cleavages. In this class cleavage is unequal. Cleavage furrows extend from the animal to the vegetal pole but at differing rates, passing faster through the animal pole cytoplasm and more slowly through the vegetal pole yolk. The first two cleavage planes are meridional and the third is equatorial but forms above the yolk resulting in an unequal cytoplasmic distribution. Thus the embryo has four smaller cells at the animal pole and four larger cells at the vegetal. The cleavage pattern is still radial holoblastic.

In **reptiles** and **birds** the enormous amount of yolk in the megalecithal zygote allows cleavage to occur only in the *blastodisc* at the animal pole. Cleavage furrows appear on the blastodisc but do not penetrate the yolk. A single-layered *blastoderm* is initially produced; then equatorial cleavages divide the cells into a layer three to four cells thick. The yolky eggs of most fishes also develop in this way. As the zygote is not completely cleaved in these animals, cleavage is termed *meroblastic* and, in the case of reptiles, birds and most fish where only a blastodisc is present, cleavage is *discoid*.

Mammalian cleavage is very different from the other types of cleavage described. Mammalian zygotes are very small (average diameter 100 μm) being miclecithal due to the *secondary reduction* of yolk associated with viviparity. Despite this reduction in yolk, a polarity can be identified in the oöcyte prior to fertilization, by the eccentric position of the spindle and a lack of microvilli on the oölemma directly superjacent; this region is also the site of extrusion of the polar cells on completion of meiosis. It seems that sperm do not generally penetrate the oöcyte in this region; no symmetry appears to be conferred by sperm entry. After fertilization the male pronucleus moves through the (now) zygote cytoplasm towards the centre, as does the female pronucleus after its meiotic division is completed. Thus at the commencement of cleavage the two pronuclei are relatively central in the zygote. At present there is no firm evidence concerning the absence or presence of segregated developmental determinants in the human oöcyte cytoplasm.

Cleavage in mammals is slow in comparison to amphibians, each division being 12 to 24 hours apart (a frog zygote can divide into 37 000 cells in 43 hours). The first cleavage plane is meridional, as in other species. However, in the second cleavage only one cell divides meridionally; the other divides equatorially. Later the four blastomeres undergo rearrangement to bring the cleavage planes at right angles to each other. This type of cleavage is termed *rotational holoblastic* and appears to be peculiar to mammals (Gulyas 1975¹). The early cleaved cells do not divide synchronously, so there is often a 3-cell stage, or a 5-cell stage. Unlike most other animals, the zygote genome is activated during early cleavage in mammals so that these early cleavage planes are not controlled by maternal cytoplasmic determinants as in amphibian cleavage (see above). Following the third cleavage, i.e. at the 8-cell stage, the mammalian blastomeres, alone, undergo *compaction*, where the cells maximize their contact with each other, forming a compact ball of cells. The outer cells form tight junctions, whereas the inner cells form gap junctions. The outer cells also exhibit cellular polarity with distinct outer (polar) and inner (apolar) surfaces. The fourth cleavage division produces a 16-cell morula which has an outer layer of cells, termed *trophoblast*, destined to form extraembryonic structures, especially the placenta, and an *inner cell mass* which will in part give rise to the embryo. The trophoblast cells secrete fluid into the morula producing a space and sequestering the inner cell mass to one side of the trophoblast. The structure so formed is the *blastocyst*; it is specific to mammalian development.

Gastrulation

Gastrulation is a process in which the cells of the blastula are rearranged into new positions to form a basic multilayered body plan of the embryo. During this process large cell populations move in concert to become apposed to other initially distant cell populations. Cell populations which will be found in certain positions **within** the embryo after gastrulation can be identified **on** the blastula surface before gastrulation begins; thus the embryonic layers, ectoderm, endoderm and mesoderm, can be mapped on the blastula surface of, for example, amphibians, and the prospective regions of certain tissues can be predicted in birds and mammals (3.3² indicates the time and stage at which gastrulation occurs in chick, rat, mouse and human embryos).

Invertebrate Gastrulation

The simplest form of gastrulation entails the *invagination* of a roughly spherical, single-layered ball of cells (the *blastula*), to form a double-layered structure containing a new cavity, the *archenteron*. The initially circular rim through which invagination occurs is the *blastopore*. Sea urchin gastrulation is of this kind. In such embryos the archenteron expands forward to make contact with, and fuse with, a small depression, the *stomodeum*. In this way the 'mouth' of the pluteus larva of the sea urchin is formed. The original blastoporal opening of the archenteron remains as the 'anus' of the larval gut. In these embryos as soon as gastrulation starts mesenchyme cells form and break free from the invaginating inner wall of the *blastocoele*; these primary mesenchyme cells foster the deposition of calcite spicules which form the larval skeleton. A further population of mesenchyme cells is generated from the anterior region of the invaginating archenteron. Filopodial extensions of the mesenchyme cells assist the gastrulation process (these extensions traverse the mucopolysaccharide matrix enclosed in the blastocoele cavity and become attached to the inner wall of the blastula and by contraction drag the archenteron inwards (Gustafson & Wolpert 1961^[4]). It is important to note the association between the invagination of the archenteron and the internal proliferation of a mesenchyme cell lineage. (For details of sea urchin gastrulation see Gilbert 1991^[4].)

Chordate Gastrulation

The simplest form of this is seen in the development of *Amphioxus* (*Branchiostoma*); here, as in the sea urchin, a gastrula is formed by the invagination of a spherical blastula (3.2^[4]). However, unlike the sea urchin, the invagination process involves the movement of **defined** prospective areas of mesoderm and endoderm from the blastula surface to the interior as the archenteron is formed; also no free mesenchyme cells are proliferated into the blastocoele space. At the end of gastrulation the roof and upper sides of the archenteron are formed by mesoderm, and the rest of the cavity is lined with endoderm. At the end of gastrulation, the mesodermal (chordamesodermal) roof of the archenteron, in contact with the overlying ectoderm, induces the formation of a *neural tube*. Then the margins of the endoderm (lateral to the chordamesoderm) pass medially where they meet and fuse in the mid-dorsal line, to form a rod-like *notochord* above and the roof of the true *enteron* (*embryonic gut*) below. Simultaneously a series of mesodermic evaginations form segmental *coelomic pouches*; these expand and fuse to form the *coelom* (3.2^[4]). Although these initial stages of the development of *Amphioxus* (a prototype chordate) serve as a good model for the early development of most vertebrates it must be remembered, however, that the formation of metamerically arranged coelomic pouches is an unusual feature. Moreover, no real head structure is formed in *Amphioxus* and it has an excretory system whose basic elements, solenocytes, resemble the flame cells of flat worms (Goodrich 1930^[4]). Above all a defining feature of vertebrate development, the *neural crest*, is missing (see below); this raises the possibility of the diphyletic origin of chordates (see Lovtrup 1974^[4]).

Amphibian Gastrulation

The amphibian provides a better example of *vertebrate gastrulation*. The process involves

invagination of predetermined regions of the blastula via a blastopore which is at first crescent-shaped with only a dorsal blastoporal lip; it then accrues lateral borders becoming U-shaped; lastly a ventral border can be discerned and the blastopore presents, finally, a circular hole in the vegetative hemisphere. The amphibian blastula (generally) possesses two layers of cells over the animal hemisphere, including around the marginal zone near the equator, whereas the vegetal hemisphere is full of larger yolk-laden cells. Both layers of the animal hemisphere invaginate at the blastopore. The dynamic movements of chordamesodermal cells from the exterior to the interior via the dorsal lip of the blastopore, and of the mesoderm later via the lateral lips, are matched by a more passive inward movement of mesoderm and prospective endoderm via the ventral lip.

There are important differences in detail between the two major groups of amphibians which relate to the precise location of the prospective mesoderm and may reflect the diphyletic origin of the Class. In urodeles (newts and salamanders, e.g. *Amblystoma*) the prospective mesoderm is located on the surface of the blastula; in anurans (frogs and toads, e.g. *Xenopus*) it is located immediately beneath an outer layer of cells in an area corresponding to that seen in urodeles (Keller 1975; 1976). 3.2 presents the classic picture of gastrulation and its consequences in a typical urodele. The site of the dorsal lip of the blastopore is indicated by a small pigmented depression more or less at the boundary between the marginal zone and the yolk-laden vegetal cells; invagination to form the archenteron thus begins in the grey crescent area in a region of prospective endoderm. The invagination of the cranial endoderm is followed, progressively, by that of the chordamesoderm, and somitic and lateral plate mesoderm. This movement gradually involves the entire blastopore which is simply the morphological manifestation of the dynamic movement of cells from the surface to the interior. The invaginated chordamesoderm, which is located in the roof of the archenteron, becomes constricted lateromedially and extended craniocaudally through the intercalation of its component cells (Keller 1984). The remaining mesoderm comes to lie between the outer ectoderm and the inner endoderm which eventually lines most of the archenteron. The lateral components of the so-called 'mesodermal mantle' are arranged in the order expected from the disposition of their prospective precursors on the surface of the blastula.

The material first carried in at the dorsal lip forms the *prechordal plate* (Adelmann 1922, 1926; see also p. 147); it consists of mesoderm and foregut endoderm. The coelom, in amphibians, is formed by cavitation of the mesoderm; there are no coelomic pouches as seen in *Amphioxus*. Once the archenteron roof has been formed by the chordamesoderm its proximity to the overlying ectoderm initiates induction of the *neural plate* which rolls up and fuses into a neural tube. In amphibians neural populations are formed in the trunk and head from cells termed *neural crest*; these cells also give rise to mesenchymal populations in the head. Neural crest cells are found, prior to neurulation, between the neural plate and the surface ectoderm in all vertebrate embryos. (For a detailed account of amphibian gastrulation see Gilbert 1991.)

Reptilian and Avian Gastrulation

The presence of large amounts of yolk in the eggs of reptiles and birds not only confines cleavage to the blastoderm but either severely limits (reptiles) or completely prevents the

formation of an archenteron. At the end of cleavage in these forms the blastoderm consists of two layers of cells, an upper *epiblast* and a lower *hypoblast* which is adjacent to the yolk. The predetermined cells which formed a spherical blastula in, for example, amphibians, is now represented in two dimensions on the epiblast of the flattened blastoderm. The outward sign of gastrulation is the formation of the *primitive streak* on the surface of the embryo; this represents a progressive, craniocaudal ingression of cells along the dorsal midline. Inasmuch as it represents a dynamic structure caused by the inward movement of *prospective mesoderm* and *endoderm* from the surface to the interior, the primitive streak of amniotes is analogous to the *blastopore* of amphibians. In reptiles, a tube-like structure is formed during gastrulation by the initial invagination of cells at the cranial end of a broad primitive streak. This structure, said to be lined entirely with mesoderm, is known as the *chordamesodermal canal*; it corresponds to the archenteron of amphibians. It is less obvious in most avian embryos, although a small pit at the cranial end of the primitive streak (Hensen's node), the site at which chordamesodermal cells invaginate to underlie the ectoderm, may be regarded as a remnant.

Mammalian Gastrulation


The gastrulation movements seen in reptilian and avian embryos evolved as adaptations to eggs with large amounts of yolk. Interestingly these movements are retained in mammalian embryos even though there is a secondary reduction in the size of the eggs caused by the development of viviparity. The transformation of the mammalian embryonic cells, the *inner cell mass*, into a two-layered germinal disc composed of (as in avian embryos) epiblast and hypoblast corresponds to the end of cleavage (the blastula stage) of other vertebrate embryos. A primitive streak appears on the epiblast surface through which prospective embryonic endoderm and mesoblast cells are shifted (and/or generated) from the epiblast into their relative positions beneath the remaining surface ectoderm.



It is apparent that the production of a layered embryo by the invagination of a spherical blastula (amphibian) is superseded by a different mechanism in *blastodermic* gastrulation (reptiles and birds), some mechanisms of which are retained in mammalian gastrulation. The morphology of the cells formed by this latter ingression is different, as is the extensive production of extraembryonic structures (see below) for both in ovo development and in utero development. The final derivation of the embryo from a simple layer of epithelium, the induction of the primitive streak and the production of mesenchyme are all unlike the early types of gastrulation.

Extraembryonic Membranes—The Evolution of Amniotes

Ancestral reptiles developed a mechanism of containing highly yolky eggs within a closed environment (the cleidoic egg). Embryos developing in such an environment produced not only the ectoderm and endoderm concerned with embryonic development but also extensions of these layers (membranes) which formed around the yolk to aid the transport of nutrients, between the embryo and the egg shell to allow gas transfer, as an extension of the gut to sequester waste products outside the embryo, and around the embryo to provide an aqueous environment for embryonic development. Thus the *extraembryonic membranes* were formed.

Four fundamental extraembryonic membranes develop. The *yolk sac* and the *allantois* are continuous with, and derived from, the embryonic endoderm and splanchnopleuric mesoderm. They become associated with extraembryonic blood vessels thus producing a *vascular splanchnopleure*. When yolk is present the vascularized yolk sac encloses and ensures the utilization of the stored material. The allantois serves to store excretory waste and, with the chorion, it may form a respiratory membrane. The *amnion* and *chorion* are developed from, and are in continuity with, the embryonic ectoderm and the somatopleuric mesoderm; they form an *avascular somatopleure*. The amnion and associated chorion provide an aqueous environment, physical protection, and, after secondary vascularization, a means of respiratory exchange with the exterior. The secondary vascularization of the chorion is by co-aptation and fusion of its internal aspect, depending on the Class and Species, on some combination of: a normally expanding yolk sac; a partially or wholly inverted yolk sac; or a (highly) variably expanded allantois. The production of the extraembryonic membranes is of such evolutionary significance that reptiles, birds and mammals are collectively termed *amniotes*.


Of the **egg-laying amniotes**, many present-day reptiles, all birds, and the prototheria (e.g. the duck-billed platypus) develop extraembryonic membranes as described above. As embryonic development proceeds (3.3 ) , the amnion develops as folds from the ectodermal body wall which grow from the head, tail and lateral regions of the embryo; the folds fuse over the dorsal surface of the embryo to create a cavity into which amniotic fluid is secreted. The option of *viviparity* evolved through the retention of the egg by the mother; probably initially as a protection of the egg from predators. Subsequent loss of yolk and the consequent need to forge an efficient *physiological link* between embryo and mother led to the development of a *placenta* with components derived from the pre-existing extraembryonic structures and the maternal tissues. Metatherian mammals (marsupials) develop a yolk sac placenta in utero, based on a link between the yolk sac splanchnopleure and the maternal endometrium. However, the gestation length offered by this form of placentation is very short; consequently in these species, the young are born very immature, at a 'larval stage', with precociously developed lungs, mouth and upper limbs, allowing the newborn to climb to the pouch and attach to the teat. The hind limbs are, however, still at an embryonic stage as are other systems of the body.

Eutherian mammals exploit the allantoic circulation which is no longer needed for respiratory purposes as it is in avian embryos. The allantoic (umbilical) vessels form a vascular link connecting the embryo to a specialized region of the chorion and the maternal circulation, i.e. the placenta. The variety of placental types need not concern us here (see p. 166 ); suffice it to say that superficial implantation as seen in the pig may be regarded as the primitive condition and interstitial implantation as seen in the mouse and man the most advanced (3.3 ). Note, however, that the extraembryonic membranes and the placentae of mouse and man are utterly different topographically.

During the time of implantation the outer layer of the blastocyst, the trophoblast, shows two different cell arrangements, a cellular *cytotrophoblast* and an external invasive *syncytiotrophoblast* composed of a multinucleate mass of cytoplasm which leads the implantation into the maternal endometrium. Later extraembryonic mesoblast from the inner cell

mass forms a layer over the inner surface of the cytotrophoblast; it is this trilaminar layer which is termed the chorion.

In placental mammals the extraembryonic membranes develop **before** the embryonic tissues; they utilize new methods to ensure the survival of the embryo. The chorion in particular has different roles: it produces hormones which maintain the status of the maternal uterine endometrium; it (inter alia) produces cells which support the maternal vessels invaded by the implanting embryo; it suppresses the immune response of the mother to the implanted embryo (which bears paternal as well as maternal genes); and it affects other regions of the maternal body stimulating milk production later in development.


Because the readiness of the chorion to assume its various functions is paramount to the survival of the embryo, early development in mammals is especially related to development of the extraembryonic membranes and the establishment of the placenta. Thus the development of mammalian embryonic tissues lags behind the timescale of, for example, the chick (3.32 ). In the chick eggs are deposited and incubation commences about 24 hours after fertilization; developmental stages are related to incubation at 39.4°C. The notochord is formed after about 20 **hours'** incubation but the amnion is not formed until 60 hours' incubation. In human embryos the amnion is formed before any embryonic development has occurred, by about 10.5 **days**; the notochord forms later, at about 16 days.

Primary Embryogenesis


Gastrulation, by moving cell populations from the surface of the blastula or blastoderm to the interior, and by specifically juxtaposing the chordamesoderm to the ectoderm, sets in train a series of developmental processes initially termed the *primary inductive interaction*. Such an interaction initiates a phase of morphogenesis which establishes a *basic axial structure*, common to all vertebrates. A number of events occur more or less simultaneously. The ectoderm on the dorsal surface of the embryo, in contact with the chordamesoderm, flattens to form the *neural plate* which becomes bounded by prominent folds. These *neural folds* rise up and join in the midline to form the *neural tube* medially and other neural populations, not involved in the fusion, the *neural crest* and *ectodermal placodes*, laterally. These neural populations are the precursors of the central and peripheral nervous systems. The mesoblast beneath the neural tube forms the *notochord* along the midline (axis) and paraxially segments into the paired *somites*, which are generated in a *craniocaudal progression*. The endoderm forms a continuous layer beneath the mesoblast which proliferates and migrates laterally forming a layer between the superjacent surface ectoderm and the subjacent gut endoderm. Starting in the region lateral to the somites, the mesoblast splits into two populations separated by the *intraembryonic coelom*, with those mesoblast cells adjacent to the coelom transforming into a *mesothelium*. The region consisting of surface ectoderm, underlying mesenchyme and mesothelium (the epithelial layer lining the intraembryonic coelom) is known collectively as the *somatopleure* (with both the mesenchymal population and coelomic epithelium (mesothelium) originating in the region being described as somatopleuric), whereas the region consisting of endoderm, underlying mesenchyme and mesothelium is known collectively as the *splanchnopleure* (the mesenchymal population and overlying mesothelium similarly is termed splanchnopleuric). Segmentally


arranged *nephrogenic elements* develop at the junction between the somatopleuric and splanchnopleuric regions.


During these changes the embryo elongates and distinct trunk and head regions emerge. The rapid growth of the brain promotes head flexion and the lateral walls of the embryonic disc converge ventrally; thus a recognizable embryonic shape is formed. After head folding the gut can be delineated into fore, mid and hind portions; in amniotes the midgut is connected by a stalk to the yolk sac and the hindgut is connected by a stalk to the allantois. Later there are signs of division of the neural tube into fore-, mid- and hindbrain and about this time too, *neural crest cells* begin to proliferate and migrate internally, and away from their site of origin (at the fold between the surface ectoderm and neural ectoderm) to form neural populations in the trunk and major mesenchymal populations in the head which give rise to the *pharyngeal arches*.


At the end of this phase (3.4 ) vertebrate embryos are more alike than they are different. Despite original variations in egg size, pattern of cleavage and apparent differences in gastrulation, the end product is a remarkably similar body plan. This comparison remains valid at the beginning of *organogenesis* but from this time differences of structure soon arise as the development of the different genera diverge from the plan. These individual differences require specific study.

Embryonic Convergence

Von Baer (1825) (see p. 92 ) recognized that all vertebrates pass through an embryonic stage which is remarkably similar among the group as a whole. This stage is attained after primary embryogenesis as described above and is distinguished by the presence of the pharyngeal arches; thus it has been termed the *pharyngula stage*. It features a basic body plan with three specified axes, a midline neural tube with an expanded cephalic region, a midline heart, a midline gut, a series of paired pharyngeal arches around the cephalic end of the gut and bilateral symmetry in the lateral structures. Similarity of appearance is matched by similarity in size so that at this stage the craniocaudal dimension of most vertebrate embryos averages around 7–8 mm. There is a striking difference, however, in the time it takes embryos to reach the pharyngula stage: for instance it takes 4–5 **days** in the case of birds (developing at about 39°C), but 4–5 **weeks** in the case of mammals. A comparable stage is reached in *Xenopus* (an amphibian), developing at a much **lower** temperature of about 23°C, after only 2–3 days.

Central to any discussion of the relation between phylogeny and ontogeny (see also p. 92 ) is the fact that convergence towards the pharyngula stage is achieved despite large differences in the developmental processes which precede, and the varying time it takes to reach, this body plan. The pharyngula stage can be considered to be a period of major developmental and evolutionary constraint. This concept can be explained in terms of the interactions of embryonic cells at the various stages of development. During early development there are relatively few interactions and few domains (local areas where interactions may occur); thus the embryo is free to develop and change with few constraints. Similarly, during organogenesis, although there are many local interactions progressing as the body systems and organs develop, and many

embryonic domains, there are few interactions **between** the domains. However, at the pharyngula stage there are a number of critical cellular interactions occurring and, most importantly, the domains of these cellular interactions **must** interact. This imposes a major developmental constraint on the system, such that this stage of development is highly conserved between vertebrates. This period of maximum constraint has been termed the *developmental evolutionary hour glass* (3.4 ); it is imposed by complexity and it is the final phase during which the embryo must behave as a whole if it is to survive. During the early and later phases of development, each side of the pharyngula stage, evolutionary processes may generate change without detriment to the basic vertebrate structure; however, changes occurring at the pharyngula stage may prove lethal.

Ontogenetic change can give rise to phylogenetic change by a variety of mechanisms, such as a change of the embryonic source for forming the structure, in the embryonic processes specifying the structure (e.g. changes in pattern-generating mechanisms), in developmental sequence, and in the timing of developmental events. Even small changes in embryonic processes may result in major changes to adult morphology or function. This is the basis of saltatory evolutionary theory, whereby major morphological changes are thought to occur suddenly in the fossil record, as opposed to a more gradualistic change, which characterizes other morphological features. Development thus forms the basis for all evolutionary theory and studies. (For a detailed consideration of major associations between development and evolution see Hall 1992 .)

'All that we call phylogeny is today, and ever has been, ontogeny itself. Ontogeny is, then, the primary, the secondary, the universal fact. It is ontogeny from which we depart and ontogeny to which we return. Phylogeny is but a name for the lineal sequences of ontogeny, viewed from the historical standpoint'.

Developmental Biology—The Analytical Approach

A comprehensive analysis of embryonic development impinges on many facets of biological science, especially those concerned with cell structure and function and the molecular basis of gene action and control. Bearing in mind the cellular foundation of developmental systems this is not altogether surprising; what is sometimes forgotten, however, is that the study of the way in which embryos develop has, in recent years, initiated fundamental advances in our knowledge of gene action and cellular physiology.

The overriding problems of animal development concern the need to understand the mechanisms by which cellular diversity arises from the relatively simple structure of the egg, and how specific developmental pattern and precise morphological form is generated. These are of course problems shared with other developmental systems, notably plant development, regeneration and wound healing, and phenomena such as metaplasia, metastasis and senescence. In this context embryology is seen as part of the larger science of *developmental biology*. Concerned with the common and related problems associated with all the systems mentioned above, developmental biology has largely replaced the narrower discipline of embryology. The techniques currently

available to the developmental biologist include electron microscopy, microsurgical manipulation, immunocytochemistry and probes for specific gene expression. A battery of such techniques is often employed to study a particular problem, although it is sometimes the case that the methods used seem to lose sight of the original aim of the investigation. It is always important to ask of any study not only what is its general purpose but what precise question within its chosen field it is attempting to answer. In some cases it is also necessary to consider the ethical dimension, for instance when embryos are either being subjected to microsurgery or gene transfer experiments.

A necessary prerequisite for the experimental analysis of animal development is an accurate and detailed description of the embryos under investigation. The emergence of embryology as a distinct science coincided with the refinement of techniques associated with light microscopy, thus facilitating the precise location of embryonic tissues and their progressive fate during development. Subsequently electron microscopy greatly enhanced this cellular level of description. What may be regarded as even more refined levels of description, virtually at the molecular level, are now available through the use of immunochemical techniques and gene probes. It is a combination of such techniques with those of micromanipulation experiments, devised many years ago, that has recently led to a significantly greater understanding of, inter alia, the establishment of embryonic axes (see p. 118), the complexity of head morphology (see p. 157) and basic patterning of the head and the body region.

The generally valid picture of embryonic development which we now have is mainly the result of a detailed study of relatively **few** animals. By and large, the material was originally chosen because it was, or became, easy to obtain and observe. Latterly attention has become focused, even more narrowly, on those embryos which have not only yielded a considerable body of knowledge in the recent past (e.g. chicks, *Xenopus*, *Drosophila*, the laboratory mouse), but have proved especially well suited to current trends of research in developmental biology. This concentration on a limited number of preferred types has been the subject of criticism in the past (e.g. Needham 1959) and also more recently (Raff 1992). The renewed interest in the relation between evolution and development (Gould 1977; Lovtrup 1984; Hall 1992) highlights the value of studying a wide variety of animals.

An Historical Perspective of Experimental Embryology

The main areas of concern of developmental biologists today are inextricably linked with those topics which were of compelling interest to the embryologists of the past: How does the egg give rise to the embryo? What is the basis of cellular differentiation? What factors are involved in morphogenesis? What is the relation between development and evolution? More recent efforts to refine these questions and provide satisfying answers to them are dealt with in a later section (p. 110). Here the significant earlier work which, by experimental analysis, achieved a fundamental understanding of the mechanisms involved in embryogenesis will be briefly described.

Many of the techniques described below, those of micromanipulation, grafting, translocation and



deletion of fragments of embryonic tissue, together with necessary improvements in the general methods used to maintain the experimental material, were devised during the first half of the 20th century. The end of this period saw the beginning of a newer phase of experimentation involving nuclear transplantation and more refined methods of tracking the movement and disposition of embryonic cells.

Preformation and Epigenesis

The explanation of how the apparently simple egg generates the obviously complex embryo was the source of a major controversy over several centuries. On one side were those who supported *preformation*; they believed that the egg contained a miniature adult form and that development was essentially a process of growth of a pre-existing structure. On the other side were the exponents of *epigenesis* who maintained that the embryo developed gradually from a relatively simple structure, analogous in some ways to the opening of a leaf or flower bud of a plant. Harvey (1578–1657), famous for his discovery of the circulation of the blood, was an early supporter of this epigenetic view, based on his observations of chick development. Harvey's dictum 'ex ovo omnia' now appears to be remarkably foresighted; more practical was his view that 'eggs cost little and are always and everywhere to be had'. Among preformationists controversy raged between the ovists, who believed that the egg contained the preformed embryo, and the spermists, who favoured the sperm. There was, in fact, dating from the time of Aristotle, a strong presumption to imagine semen as the active principle initiating development in a passive, featureless egg which merely provided the material for embryogenesis. In a male dominated society the spermists prevailed to such an extent that some of the early microscopists were convinced that they could actually detect homunculi in human sperm and drew some imaginative figures. Eventually with more careful, and less biased, observations the epigenetic view gained general acceptance. It can be maintained, however, that inasmuch as the genome contains essential molecular information for specific development and that many eggs contain predetermined cytoplasmic components, a preformationist input remains a valid concept. In fact, the two views are no longer regarded as incompatible; indeed the 'modern view' is that they are not only complementary but inseparable (for a recent discussion, see Hall 1992^[1]).



Towards the end of the 19th century a few embryologists adopted an analytical approach to the study of animal development. Importantly some simple experiments on the earliest (cleavage) stage of development eventually provided conclusive evidence against the idea of preformation. Initially, Roux (1888^[2]) demonstrated that destruction of one of the first two cells of the frog embryo produced the equivalent of half an embryo. Later work, however, which involved the complete separation of the first two cells gave one of two results: either two complete (half-sized) embryos were formed or one of the separated blastomeres gave a complete embryo and the other simply a mass of apparently undifferentiated cells (3.5^[3]). The striking disparity between the results of the separation experiment depends on the relation of the first cleavage plane to the grey crescent which is formed at fertilization (see p. 98^[4]). If the first cleavage **bisects** the grey crescent **two** complete embryos are produced; in a cleavage which confines the crescent to one blastomere only, and thus deprives the other of this feature, then only the former produces a complete embryo. The importance of this structural element is discussed below in

relation to the discovery of the 'organizer'.


A complementary set of separation experiments, involving both 2- and 4-cell stages, was carried out by Hans Driesch (1891) using sea urchin eggs; the separated blastomeres produced half and quarter size larvae (3.5 ). Although Driesch claimed that even smaller larvae were obtainable from separated blastomeres of the 8-cell stage, subsequent work did not support this finding. Driesch's experimental results supported his theoretical view of development which contained an element of vitalism, now regarded as unacceptable. Driesch (1921 ) considered that every part of the egg cytoplasm was equipotential and that therefore the formation of an increasingly complex embryo was not possible unless this basic material was organized by a non-material agency; he called this organizing principle *entelechy*. This concept, derived from the Greek word meaning soul, was central to the philosophy of Aristotle (384–322 BC). It was in fact a key element in Aristotle's own view of animal development which he viewed as a succession of linked material events the organization of which was dependent on, and driven forward by, the non-material and all pervading *soul*.

Blastomeric Potential


Both the amphibian and sea urchin investigations demonstrate that the initial blastomeres have the potential to form whole embryos; they thus possess a considerable capacity for regulation. They are not highly structured, confirming that in the original restricted sense there is no basis for preformation. Some eggs (ascidians, annelids and molluscs) are, or at fertilization become, highly structured. Separation of the early blastomeres of such 'mosaic eggs' give embryonic fragments which are compatible with a strictly determined cell lineage, but even in these cases it is possible to demonstrate some regulation of structure.



The first two cleavages of the sea urchin egg are meridional, are at right angles and, significantly, they do not cut across, but include, the animal–vegetal axis of the egg. The importance of this axis was highlighted by Hörstadius (1939 ) who demonstrated, for instance, that separation of the upper and lower halves of the 8-cell stage (cutting across the animal–vegetal axis) produced not half-sized complete larvae but two quite different but derived embryonic structures (3.5 ). Hörstadius suggested that a balanced interaction between animal and vegetal components of the egg was essential for the harmonious development of the larval structure and proved his hypothesis by an elegant series of combination experiments involving partial fragments derived from the animal and vegetal halves taken from the early cleavage stages of the embryo.







Numerous investigations have since focused attention on the importance of the *intrinsic axial structure* of the egg which becomes entrenched during cleavage. This has been demonstrated for many animals, and is related to the distribution of factors which appear to determine specific cell lineages (e.g. in *Xenopus* and ascidians); it also appears to underpin the linear-craniocaudal pattern of structure of the developing organism (e.g. in insects). Distinct, and experimentally demonstrable, polarity and the dependent early separation of cell lineages, is not, however, a feature of all types of eggs; notably the mammalian egg, which although similar in size and

superficial appearance to that of the sea urchin, does not possess an equivalent polar structure. In this case cell lineage determination is delayed until the compacted 8-cell stage (see p. 134 .

Nucleocytoplasmic Interaction—Nuclear Transplantation

The central concept that progressive nucleocytoplasmic interaction provides a satisfactory explanation of embryonic development became generally accepted during the early part of the 20th century. The definitive form of this concept was formulated by Morgan (1934 ); it embodied the following elements. Initially, equipotential (genetically equivalent) nuclei are distributed during development in a *heterogeneous cytoplasm*; the cytoplasmic environment then modifies the *genetic activity of the nuclei*; the altered activity of the nuclei will in turn modify the surrounding cytoplasm; such reciprocal interactions result in the progressive differentiation of embryonic cells as development proceeds. The existence of a *heterogeneous egg cytoplasm* and its capacity to modify the activity of the early cleavage nuclei accords with the experiments described above. The nuclear transplantation experiments, described below, address the problem of nuclear equivalence and the permanency of any change imposed by the cytoplasmic environment.

By applying a hair loop constriction to a newly fertilized newt egg Spemann (1914 ) carried out what was in effect an in ovo nuclear transfer (3.5 ). The constriction confined the zygote nucleus to one half of the egg but did not prevent its division into 16–32 cells. At this stage one of the cleavage nuclei was allowed to enter the anucleate portion of the egg; cleavage followed in this portion and in some cases a second, twin, embryo was formed. Although, as would be expected, the relationship of the constriction to the grey crescent (see above) affected the outcome of the experiment it clearly demonstrated the *equivalence* of the early cleavage nuclei in amphibian embryos. This means that, provided they are not damaged by the experimental procedure, **any** of these nuclei can, in collaboration with the egg cytoplasm, programme the whole of development. Do nuclei retain this capacity beyond the early cleavage stage? Amphibian nuclear transplant experiments provided an unequivocal, and positive answer to this question.

Initially the technique for the isolation and physical transfer of animal cell nuclei was established using amoebae (Commandon & DeFonbrune 1939 ; Lorch & Danielli 1950 ). Using a frog (*Rana pipiens*) a similar technique was used by Briggs and King (1952 ) to transfer nuclei from blastulae and gastrulae to enucleated eggs. Variable results were obtained, including a variety of abnormal embryos, but importantly, a few embryos went on to develop completely normally and to produce viable tadpoles (3.5 ). A significant extension of this work involved the serial transplantation of nuclei from the blastula to neurula stages obtained from an initial transplant (King & Briggs 1956 ). The derived clones showed persistent, and similar, patterns of normality, including some which produced a high percentage of normal embryos. Clearly a proportion of embryonic nuclei remained totipotent even in cells committed to differentiation. Using the South African clawed frog (*Xenopus laevis*) and a different technique involving ultraviolet radiation to enucleate the eggs, together with a genetically determined nuclear marker, to distinguish host from donor nuclei, Gurdon (1960 ) and his colleagues confirmed and considerably extended the original work, most notably to show that nuclei derived from the



intestinal epithelium of feeding tadpoles could produce a few viable embryos following serial transplantation (Gurdon 1962^[4]). Even transplanted and serially transferred nuclei, derived from the keratinized cells of adult *Xenopus* epithelium have been used to produce living, but eventually moribund, tadpoles (Gurdon et al 1975^[4]). It could be imagined that these experiments on amphibian embryos might provide a route for the nuclear cloning of mammals, including man. Recent work (McGrath & Solter 1984^[4]) suggests that this is not even a remote possibility.

Significance of Gastrulation




Although the dynamic nature of gastrulation and its association with the formation of mesoblast is implicit in much early descriptive work, the current view of gastrulation among chordates, i.e. one of coordinated cell movement leading to an inductive interaction between mesoblast and overlying ectoderm, and the consequent development of axial structure, derives from the experimental work carried out in the earlier part of this century. It was during this period that several important microsurgical procedures and cell marking techniques were developed.

The use of vital dyes (e.g. Bismarck brown, neutral red) applied to the surface of the amphibian blastula enabled Vogt (1926^[4]) to demonstrate that well-defined areas moved from the surface to the interior during gastrulation. In particular there was an active movement of prospective chordamesoderm over the dorsal and lateral lips of the blastopore during the formation of the archenteron and a more passive dragging in, ventrolaterally, of prospective endoderm (3.6^[4]). Graper (1929^[4]) demonstrated an apparently analogous shift of material from the epiblastic surface of the early chick embryo, using a combination of vital dyes and time lapse cinematography; this pioneering work was refined at a later date, particularly by Spratt (1946^[4]), using early chick blastoderms cultured in vitro. Such techniques initiated the detailed mapping of presumptive (prospective) areas (destined to give rise to particular tissues) on the surface of the chordate blastula or its blastoderm equivalent. Gastrulation was visualized as the process which ensured that these areas moved into the right place at the right time during this fundamental stage of embryogenesis.


The importance of gastrulation as the essential prerequisite for the generation of the characteristic axial structure of chordates was revealed by work which led to the discovery of the *organizer* associated with the dorsal lip of the blastopore of amphibian embryos. The fundamental contribution was made by Spemann (1918^[4]) who showed that when a small area taken from the blastoporal region of a newt embryo was grafted into another location (e.g. opposite the dorsal lip) it produced a *secondary embryonic axis*. As the amount of grafted material was small compared to the size of the induced structure it was clear that both the graft and the host tissue participated in its formation; in other words the graft organized the new structure. Elegant proof that this was the case was provided by using a *xenoplastic grafting* technique where the dorsal lip from **one species** of newt was grafted into the early gastrula of **another species** whose cells were distinguished by their pigment content (Spemann & Mangold 1924^[4]) (3.6^[4]). The discovery of the organizer opened up many (mostly productive) areas of research, including the discovery of an analogous region (Hensen's node) in the primitive streak stage of the chick embryo (Waddington 1932^[4]). A fruitless search for the chemical nature of the organizer eventually led to the discovery that even inorganic material could induce neural

structures in gastrular ectoderm. Above all, however, it was the work of Spemann and others which established the concept of *primary embryonic induction*, namely, that it is the **contact** between the archenteron roof and overlying ectoderm, brought about by gastrulation, that **induces** the formation of the neural (medullary) plate and the corresponding axial organization of the mesoblast which lies beneath the prospective central nervous system (CNS). This is the major consequence of the complete invagination of the presumptive chordamesoderm which commences with the appearance of the dorsal lip of the blastopore in amphibian embryos or its equivalent in other chordates. 3.6  illustrates some relatively simple experiments which demonstrate what is undeniably the most significant interaction of embryonic tissues during the development of vertebrate animals. (For a detailed account see Saxén & Toivonen 1962 .)

Anamniote Induction of Mesoderm

Although the significance of the Spemann organizer in the formation of the chordate embryo cannot be underestimated it has been realized for a number of years that the associated primary induction, appropriately named at the time, is preceded by another fundamental inductive event which **predetermines** the basic mediolateral pattern of the prospective mesoderm. This concerns the interaction which takes place at the boundary between the vegetal yolk mass and the marginal zone (prospective endomesoderm) at the blastula stage of amphibian embryos. This concept of the early induction of mesoderm by the underlying vegetal cells, primary endoderm, was suggested by the work of Nieuwkoop (1973 ). The pattern of the induction is related to bilateral symmetry imposed on the amphibian egg (formation of the grey crescent) at fertilization; this leads to the distinction between a ventral influence (determining blood, mesothelium, etc.) and a dorsal influence (determining notochord and somites). A review by Nieuwkoop (1985 ) provides a valuable summary of the origin and importance of the inductive interactions during amphibian development. A similar mechanism to *mesodermal induction* may explain the influence of the *hypoblast* on the *epiblast* of amniote embryos (see p. 143 ). The demonstration of a key role for the vegetal domain of the egg (or a derived structure—the hypoblast) in chordate embryos relates to the well-known vegetal influence on early development, demonstrable for the eggs of several marine molluscs and annelids (Wilson 1904). There has been a resurgence of interest in mesoderm induction following the implication of growth factors.

Organogenesis and Embryonic Tissue Interactions

In vertebrates the precursors of individual organs begin to make their appearance soon after the end of gastrulation and the subsequent formation of neural tube. The development of many of these systems is largely dependent on embryonic tissue interactions. In principle these so-called *secondary* and *tertiary inductions* resemble the primary induction inasmuch as they are dependent on the competence of the target tissue to respond to the inductive stimulus provided by a neighbouring one. The importance of these interactions is indicated in those sections which deal, in detail, with the development of specific organ systems in the human embryo; current views on the cytological and molecular basis of the mechanisms involved are also dealt with in a following section (see p. 110 .

Of historical interest are the following significant discoveries: the influence of the developing optic cup on the formation of the lens in amphibian embryos (Spemann 1901^[1]; Lewis 1905^[2]); the role of the ancestral pronephric duct in the induction of mesonephric tubules (Waddington 1938^[3]) and the essential role of mesenchyme cells in limb formation.

Significance of the Neural Crest

Not all mesenchyme is derived from mesoderm invagination through the blastopore (amphibians) or mesoblast ingression through the primitive streak (amniotes). The most important alternative origin is the neural crest. The significance, migration and fate of the neural crest cells has been (His 1879^[4]; Katschenko 1888^[5]; Platt 1894^[6]), and is currently, of great interest (see p. 240^[7]). The importance of the neural crest cannot be underestimated; it appears to be a unique feature of vertebrates distinguishing them from their chordate ancestors (Gans & Northcutt 1983^[8]).

Pioneering experiments on amphibian embryos (Landacre 1911^[9]; Stone 1922^[10], 1926^[11]; Du Shane 1935^[12]) tracked the migration of the neural crest cells by excision and translocation of their sites of origin in the crest, established the distinction between *head* and *trunk crest cells*, and gradually defined the extent of the neural crest contribution to vertebrate development. Eventually detailed mapping of the head neural crest in urodele embryos enabled the origin of the branchial structures to be determined with great precision (Hörstadius & Sellman 1946^[13]). Examples of these early experiments, which contributed to a fundamental understanding of the significance of the neural crest, are illustrated in 3.6^[14]. A detailed account of this work is given by Hörstadius (1950^[15]).

Current Concepts in Developmental Biology

Generation of Cell Diversity

Originating from a single but highly specialized cell, the zygote, the *generation of cell diversity* is an intrinsic feature of animal development. In mammals the adult organism contains in the region of 300 distinct cell phenotypes characteristically arranged to form specific organs and tissues. As in all animals above the level of sponges and coelenterates these cells may be classified according to function such as muscle, nerve, connective tissue and so on. In general, the lower the grade of organization the fewer cell types there are: coelenterates contain fewer than ten cell types, some of which perform a dual function, for example musculo-epithelial cells. Within a major animal group, such as the amniotes, a great variety of form and function is derived from similar embryonic cellular ingredients possessing broadly equivalent potential to develop into a fixed number of cell types. Any general theory of development must attempt to understand the factors causing the divergence of cell fate and the individual and collective role of cells as their phenotypes diverge; it further needs to acknowledge the constraints that evolution has apparently placed on the essential contribution of cells.

To this end it is important to appreciate the concept of a limited number of *basic cell types* from which all current diversity appears to be derived, both in development and in evolution (Willmer 1960; Lovtrup 1974). Observations on cultured cells derived from a variety of tissues led Willmer (1960) to the view that only three or four fundamental cell types existed. Based on their appearance and behaviour in culture, together with their potential to generate further diversity, he termed these cell types *amoebocytes*, *epitheliocytes*, *myxoblasts*, and *myoblasts* (myxoblasts and myoblasts were thought to be variants of *mechanocytes*). Nerve cells were not included in the original classification. Such cell types are characteristic of the initial stages of development; for instance, mesenchyme cells (myxoblasts) and embryonic epithelia (epitheliocytes) feature prominently in the gastrulation process (see pp. 96 and 142). Lovtrup (1975, 1983, 1984) has refined and expanded the notion of basic cell type in an attempt to construct a logical and cell-based framework for epigenesis. His analysis emphasizes the importance of cytoskeletal elements (e.g. microtubules, microfilaments) and the role of extracellular material specifically associated with each of his proposed 'cell orders'. To avoid a perceived ambiguity in Willmer's classification, Lovtrup proposed an alternative nomenclature for the basic cell types. *Solocytes* (*s-cells*) are those cells which are capable of free movement and do not readily form stable aggregates; as they move they may form either lobopodia (solo-lobocytes, sl-cells, i.e. amoebocytes) with short actin filaments or very long filopodia (solo-filocytes, sf-cells, i.e. myxoblasts) which contain cytoplasmic microtubules. *Colligocytes* (*c-cells*) are cells which, through their adhesiveness, form aggregates, typically epithelial; they also have two varieties, colligo-lobocytes and colligo-filocytes; the former contain microfilaments, the latter microtubules, corresponding to comparable s-cells. Solocytes produce extracellular matrix molecules, with heparan sulphate and hyaluronate being characteristic. (Lovtrup notes that hyaluronate is produced in the embryo particularly by mesenchymal cells; see also p. 153.) Solocytes form solid aggregates stabilized by short filopodia which form tight junctions with their neighbours. Colligocytes on the other hand produce a layer of reticular and fibrillar collagen within a matrix containing sulphated proteoglycans; they are adhesive and form junctional complexes. A limited number of transformations are possible between these conceptual cell types; namely solocytes can form aggregates (s-c transformation) and lobocytes can convert to filocytes. Ciliated or flagellated variants of each basic type are possible, giving a total of eight types altogether (3.7). Based on the analysis of the structure and function of this limited number of cell types, and paying due regard to the constraints imposed by evolution, Lovtrup provides a logical approach not only to the problem of cell diversity but to morphogenesis in general. (Despite the unusual terminology Lovtrup's views merit close attention, not least because they eliminate the need to relate the discussion of cell diversity to germ layer theory or to use terms which no longer seem appropriate to modern developmental biology. To quote Lovtrup (1983): '... it is difficult, or even impossible, to interpret and understand the processes of cell differentiation occurring in the embryo on the basis of terminology currently employed in cell biology'.)

Embryonic Cells and Tissues

The first cell divisions of an embryo are cleavage divisions, which repeatedly reduce the size of the cells (blastomeres), restoring the nucleus to cytoplasm ratio and resulting in cells of typical size. The arrangement of the cells within the morula produces differences between the cells

because of their relative position, their exposure to the environment and their junctional connections to other cells. This results in the *differential* expression of cell morphology: for example the outer cells of the morula become polarized and exhibit apical microvilli; they acquire different junctional connections with their lateral neighbours compared to their connections with the cells in the centre of the morula; the outer cells act in concert as a cell population forming the earliest epithelium.


After gastrulation the arrangement of embryonic cells into tissues is apparent. Epithelial cells (Lovtrup's colligocytes), which form the upper and lower layers of the embryo, have apical-basal polarity, narrow intercellular clefts, juxtaluminal junctional complexes and a developed basal lamina composed of extracellular matrix proteins synthesized by the cells. The cells between the inner and outer epithelial layers are mesenchymal in arrangement. Mesenchymal cells (Lovtrup's solocytes) have no polarity; they have junctional complexes which are not juxtaluminal and they produce extracellular matrix molecules and fibres from the whole cell surface.

These two embryonic cell states are not necessarily immutable and *transition from epithelia to mesenchyme* and vice versa occurs during development. The causative factors of such changes in cell aggregation and contact are not clear and many different factors of a temporal or locally inductive nature may be involved. Transitional events during gastrulation and the early stages of development may be different from those occurring at the later stages of organogenesis. For example, it is likely that early mesenchymal populations are heterogeneous, containing migrating epithelial cells which **temporarily** express a mesenchymal appearance: the first cells to ingress at the primitive streak and migrate between the epiblast and hypoblast form epithelia at their destination (Bellairs 1987) in the epithelial somite (see p. 144) and the somatopleuric and splanchnopleuric coelomic epithelia (see p. 155). Similarly, splanchnopleuric mesenchyme forms endothelia generally throughout the embryo but may engage in this transition either early or late. However, the nephrogenic mesenchymal cells, produced from proliferation of the coelomic epithelium, form epithelial nephrons only during a specific time period.

Conversely, epithelial cells can reorganize their extracellular matrices and transform into mesenchymal cells. This is seen in the most basic manner at the primitive streak and later in the formation and migration of the neural crest (Greenburg & Hay 1982; Hay 1989). Often cell proliferation of particular cell lines occurs at an epithelium, for example formation of intermediate mesenchyme from the proliferating coelomic epithelium and production of myogenic cells from the epithelial plate of the somite. However, a later specific example of transition from epithelium to mesenchyme occurs in the heart where endocardial epithelial cells become cardiac mesenchyme at the atrioventricular canal and the proximal outflow tract of the heart (see p. 300). This latter example is a locally induced transformation and different from the production of mesenchymal cells from a germinal epithelium.

Developmental Hierarchy

Much of the specification of the basic embryonic body plan is the result of a **hierarchy** of developmental decisions at different developmental times. The earliest embryonic cells may be described as *totipotent* meaning they have the capacity to become any cell of the adult body. (An alternative view is that the earliest cells are optimally differentiated for the stage of development they have reached, as they will be at any stage throughout development.) During development, cells respond to intrinsic or extrinsic cues by following a developmental pathway which will result in the *commitment* of those cells to a particular fate. In mammals, for instance, an early choice is made at about the 8-cell stage when some cells become committed to develop into extraembryonic tissues and others into embryonic tissues. Similar *binary choices* subsequently occur for both the extraembryonic cells and the embryonic cells. For the extraembryonic line, trophoblast cells become either cytotrophoblast or syncytiotrophoblast; the cytotrophoblast cells become either mural or polar trophoblast. For the embryonic line, inner cell mass cells become either hypoblast or epiblast; the hypoblast can become either visceral or parietal. (It is worth noting at this point that there is little to distinguish blastomeres before the 8-cell stage and few choices may be made before this time. At the time of compaction the outer cells of the morula express cell adhesion molecules, form junctions and exhibit apical microvilli. The connection of these cells results in a different environment for the cells in the centre of the morula. Thus different fates for the inner and outer populations of blastomeres can now be linked to cell signalling, interaction and response.)

3.37  shows the binary choices for particular early extraembryonic and embryonic cell fates. Within the embryo these choices focus from the general (e.g. ectoderm becomes neural plate, neural crest and surface ectoderm) to region specific (e.g. the latter cell populations become regions of the brain and spinal cord, regions of the autonomic nervous system or parts of the face and skull and epidermis respectively). Finally the tissues of the regions so formed develop very specifically into differentiated cell phenotypes, for example neurons, glia, osteocytes, fibrocytes, melanocytes, keratinocytes, etc.

Restriction and Determination

As cells become committed to a particular fate they lose the ability to choose an alternative range of developmental pathways, i.e. they become *restricted*. The term restriction is usually used to refer to limitations in the ways that a population of cells can develop. Once restricted, cells are set on a particular pathway of development and after a number of binary choices (i.e. further restrictions) are said to be *determined*. Determined cells are programmed to follow a process of development which will lead to *differentiation*. The determined state is a heritable characteristic of cells; it is the final step in restriction. After a cell has become determined it will progress to a differentiated phenotype providing the environmental factors are suitable. For example, melanocytes normally express the black pigment melanin; for this they require the presence of tyrosine in the environmental medium. While the tyrosine is present the cells achieve their differentiated phenotype with black coloration. If, however, the cells are maintained in a tyrosine-free culture medium the cells can no longer synthesize melanin and they become pale; they no longer appear differentiated. If at a later time the tyrosine is replaced in the medium the cell line can once more synthesize melanin. This demonstrates that the *determined state* is *stable*

and not dependent on the environment, whereas the *differentiated phenotype is labile*. This process is evident in the repair of a wound or fractured bone in an adult, as well as in an embryo.

The process of determination and differentiation within embryonic cell populations can be assessed by the ability of cell populations to produce specific proteins. All cells have a series of genes which code for proteins considered essential for cellular metabolism. Such genes and proteins have been termed *primary* (colloquially termed housekeeping genes and proteins) to illustrate their ubiquity. As cells become determined they synthesize proteins specific to their state of determination. These are termed *secondary* proteins, for example liver and kidney cells but not muscle produce arginase. At the most differentiated state cells produce *tertiary* proteins, those which no other cell line can synthesize, for example ovalbumin in oviduct cells or haemoglobin in erythrocytes (the genes and proteins in this case have been colloquially termed *luxury* to denote their speciality). Primary, secondary and tertiary proteins are an expression of stages of determination and differentiation, coded by a range of genes. Other gene products can be detected which, by their expression, *confer* a particular determination and differentiation pathway on undetermined, or even differentiated cells, i.e. establish a cell *lineage*. The genes coding for these products, which can *direct* the fate of cells faced with a binary choice, were termed 'switch genes' by Waddington (1940^[4]); they have since been identified. Their expression in cells allows two choices: the presence or absence of the gene product determines which lineage the cells generate. In vertebrates the *MyoD-1* (myoblast determination 1) gene found in myoblasts can change differentiated adipose cells, fibroblasts or hepatocytes transfected with the gene into a myocyte lineage.

Determination Pathways

As populations of cells become progressively determined they can be described as *transient amplifying cells*, *progenitor cells*, *stem cells*, and *terminally differentiated cells*.

Transient Amplifying Cells

These are cells proliferating and producing equally determined cells; they undergo *proliferative cell mitoses*. At some stage transiently amplifying populations will, as a result of an inductive stimulus, enter a *quantal cell cycle* (Holtzer et al 1972^[5]). The cells, as a response to molecular signalling, undergo a *quantal mitosis* resulting in an increase in the restriction of **their progeny**, which continue to undergo proliferative mitoses at a progressive level of determination. The quantal mitosis corresponds to the time of binary choice (see above) when the commitment of the progeny is different from the parent.

Progenitor Cells

Progenitor cells are already determined along a particular pathway; they may individually follow that differentiation pathway or may proliferate producing larger numbers of similarly determined progenitor cells which subsequently differentiate. Examples of progenitor cells are neuroblasts or myoblasts.



Stem Cells




Stem cells are cells which individually, or as a population, can **both** produce determined progeny **and** reproduce themselves. It is generally thought that whereas proliferative cell division may be symmetric, giving derived cells with identical determination, stem cells undergo asymmetric divisions, whereby one daughter remains as a stem cell (i.e. retains the determination of the parent cell), while the other proceeds along a differentiation pathway (possibly leading to death). Stem cells are seen later in development and in adult life. It appears that differing combinations of growth factors can either maintain cells in a stem cell-like state, or cause them to differentiate.

Terminally Differentiated Cells

By their extreme specialization these cells can no longer divide, for example erythrocytes and neurons.

Programmed Cell Death or Apoptosis

This is a particular variety of terminal differentiation where the final outcome is the death of individual cells or cell populations. Apoptosis is an effective mechanism for eliminating unwanted cells which die without rupture of the lysosomes and autolysis which would release their contents into the extracellular environment and cause inflammation. Programmed cell death is seen in the developing limb where cells die along the pre- and postaxial limits of the apical ectodermal ridge so limiting its extent (p. 290 ) , and similarly between the digits allowing their separation (p. 290 ) . Within the nervous system neuroblasts which project to abnormal targets and fail to receive the appropriate neurotrophic factors undergo programmed cell death.

Work on the nematode *Caenorhabditis elegans* has suggested that the cell death programme is normally **on** in all cells, and that cell death is prevented by an over-riding 'survival' programme (reviewed in Raff 1992 ) . This survival programme often involves growth factors and highly conserved gene products, such as that encoded by *BCL-2* (Tomei & Cope 1991 ) , Jacobson & Evan 1994 ) . The idea that all cells are critically dependent on survival signals, such as those generated from growth factors or the extracellular matrix, is an attractive hypothesis. It provides a simple mechanism for eliminating cells which end up in abnormal locations during development. As different tissues might be expected to produce different sets of survival factors, a misplaced cell deprived of the specific signals it requires for survival would die. It is suggested that dependence on such survival signals may be a useful mechanism for controlling cell numbers in higher vertebrates, if cells are forced to compete with one another for limited amounts of such signalling molecules.

Measurements of Determination

Examination and experimental perturbation of embryos of different stages allows the investigation of states of restriction and determination attained by particular cell populations. The addition of dyes or markers to cells within the living embryo, removal of local cell

populations, growth of portions of embryonic tissue in culture, transplantation of portions of embryo to different places in the same embryo (homoplastic), or to different embryos (heteroplastic), recombination of embryonic tissues within and between Classes (xenoplastic) and the formation of chimeric embryos (especially quail–chick, see p. 221📖) all add information about the time at which, and the position in which, cell populations become determined.

📖The addition of dyes or markers to cells and cell populations allows their relative migrations to be followed. From the movements of labelled cells and the differentiation pathways they follow, a predictive *fate map* can be produced for a known stage in development (3.42👁️). This method was used to examine gastrulation in the amphibian embryo (Vogt 1929📖; see p. 103📖) where vital dyes, added to populations of cells prior to gastrulation, permitted the visualization of their ingression at the blastopore. More recently the fate of much smaller populations of epiblast cells were demonstrated after ingression through the avian primitive node (Selleck & Stern 1991📖; see p. 143📖). The production of fate maps formed the basis of further experimentation in which regions of embryos (with a known fate) could be killed, removed, or transplanted to a different location, and the resultant differentiation of the cells could be compared to the normal developmental pathway elucidated by the fate map.

If cell populations of predicted fate are cultured in a neutral medium (i.e. one with no known inductive substances) they will differentiate. However, the final differentiation state may be different from that predicted from the fate map. The explantation and culture reveal an original state of commitment termed *specification*. The degree of specification of cell populations may differ from their determination because cell populations may be altered in the embryo by later inductive influences. However, these influences may need to be local and constant to increase restriction and determination in a cell population.

Clonal analysis is a special form of fate mapping in which a single cell is labelled. Subsequently at a later stage both the position and state of determination or differentiation of its progeny are identified (Slack 1991📖). Clonal analysis has been used to examine the fate of epiblast during gastrulation in the mouse (Lawson et al 1991📖; see p. 143📖) and to establish the degree of cell determination.

Developmental Regulation

The concept of developmental regulation emerged from numerous experiments in which individual blastomeres were removed from embryos to see if they could independently produce a normal individual. If cytoplasmic segregation occurred as the zygote divided, then individual blastomeres would possess different cytoplasmic constituents and their fates would differ. This was the basis of *mosaic development*, where the early blastomeres have a strictly determined lineage based on their cytoplasmic constituents (see p. 141📖). Such embryos can never produce monozygotic twins because the cytoplasm of the zygote is regionally restricted even before the 2-cell stage.

However, the individual blastomeres of some embryos will each produce entire embryos if separated at an early stage, showing that instead of developing into a predicted embryonic part, each blastomere could regulate its development to produce a complete organism; this is termed *regulative development*. The phenomenon of regulation exhibits *global features* wherein a mass of cells reorganizes **as a whole**, suggesting avenues of cell-cell communication for sharing this meta-organizational information and plan.

The effects of regulation are well established but the mechanisms underlying such regulation are not yet understood. There is a strong presumption that the embryo, by some mechanism, has an indication of its own size and how big it should be for a particular developmental stage. Homeostatic mechanisms are deployed in order to seek and maintain this 'target size' in embryos where naturally occurring or experimental perturbations cause it to depart from the normal growth curve. Although extreme, this viewpoint is supported by observations on twinning and fusion experiments, catch-up growth and responses to inductive reprogramming (teratogenic insults).

Twinning Experiments

When first formed, twin embryos will be approximately half the normal size, as the process of twinning leads to the formation of two embryonic axes within a normal-sized blastocyst. Interestingly, the proportions of parts within these miniature embryonic primary axes are normal, and despite starting out at half the size, each fetus is of comparable size to a singleton fetus by the second trimester of pregnancy. Twin fetuses thus go through a phase of accelerated growth during most of organogenesis although the mechanism by which this occurs is obscure. Monozygotic twins constitute a high risk group for susceptibility to malformation; although this may be due to their small initial size, it seems more likely that the risk is generated by the higher than normal growth rate that they experience at critical times in their early development .

The phenomenon of twinning reveals important insights into aspects of normal development (Slack 1991^[4]). First, it excludes any model for regional specification based solely on the localization of determinants in the egg cytoplasm. Second, it shows that these cellular interactions are able to accommodate a change of *scale* in the pattern generating mechanism. Boundaries which would be formed 100 μm apart in the normal embryo will be formed approximately 79 μm apart in the twins (Slack 1991^[4]). Third, as alluded to above, twinning clearly demonstrates that the final size of an embryonic structure does not depend on the size of the original primordium, but rather on some, as yet unelucidated, mechanism which can stop growth, when a certain absolute size has been reached.

Fusion Experiments

The converse of twinning is the fusion of two or more embryos to give one giant early embryo, or the increase in size of an embryo by the addition of cells injected into the blastocyst cavity. In such cases normal development ensues, and has been reported for aggregates of 16 8-cell mouse embryos. When born, these chimeric embryos are of normal size. The downward regulation in

size of the embryo aggregates occurs shortly after implantation, between the appearance of the amniotic cavity and the primitive streak. The mechanism of size regulation seems to involve a lengthening of the cell cycle during this period. Size regulation in these circumstances seems to be completed before the onset of organogenesis which is thus normal. The implications for normal development from these fusion experiments is similar to those outlined for twinning.

Catch-Up Growth

A region or regions of the embryo can be removed at varying stages of development without disturbing the pattern or the proportions of the fully formed embryo. This *defect regulation* involves a number of complex processes, including compensatory catch-up growth. Although this may result in reconstitution of the deleted part, later disturbances in embryonic timing and cell division patterns may give rise to abnormalities, often at locations different from the site of the primary insult (McKee & Ferguson 1984^[4]).

Inductive Reprogramming

In this example of developmental regulation, if a signalling centre, for example an apical ectodermal ridge of a developing limb, or the zone of polarizing activity in the limb bud, is grafted to an abnormal position, it can cause the surrounding tissue to follow a pathway of development which does not correspond to the fate map and is instead induced by the grafted signalling tissue (see p. 290^[5]). This mechanism is seen in experimental epithelial/mesenchymal recombinations (see below). A similar outcome can be achieved by the topical application of morphogens (see below), such as retinoic acid.

Regeneration

It is important not to confuse embryonic regulation with regeneration in adult or fetal tissues (Slack 1991^[6]). Regeneration involves the re-establishment of *regional differences* in the newly formed replacement parts, while regulation involves the re-establishment of a fate map on a partial domain of uncommitted tissue (Slack 1991^[6]). Regeneration occurs in many lower organisms, for example the formation of new heads and tails in transected flat worms, new apical and basal regions in transected hydra, or the replacement of a whole severed limb or tail, or appropriate part thereof, in adult amphibia. In general, regeneration follows one of two main types: *morphallaxis*, in which the whole re-forms by rearrangement and differentiation of the existing tissues without further growth, and *epimorphosis*, in which there is new growth of blastemal tissue, which subsequently matures into the full regenerate. Regeneration involves similar positional signalling cues to normal development, and may share some similar morphogens, for example retinoic acid and its receptors (Brockes 1996^[7]; see p. 116^[8]). An interesting form of embryonic response which is midway between embryonic regulation and regeneration is the phenomenon of scar-free dermal embryonic wound healing (Whitby & Ferguson 1991^[9]).

Cell and Tissue Interactions

It is clear from the existence of regulation and the differing differentiation pathways of cells in the early embryo, that interactions take place between the cells of the developing embryo. These cellular interactions provide the developmental integration and fine control necessary to achieve tissue specific morphogenesis. In the early embryo, such interactions may occur only if particular regions of the embryo are present, for example signalling centres or organizers (see p. 105 [1]). As the embryo matures, so interactions tend to occur between adjacent cell populations, for example epithelium and mesenchyme, and later between adjacent differentiating tissues, for example between nerves and muscle or muscle and skeletal elements. Tissue interactions result in changes or reorganization of one or both tissues; these changes would not occur in the absence of the tissue interactions. The process of tissue interaction is also called *induction*; one tissue is said to induce another. The ability of a tissue to respond to inductive signals is called *competence* (Waddington 1940 [2]). (Competence may be considered as a subset of *potency*. Potency is the total of all things a particular region of embryonic tissue can become if put into the appropriate environment. Competence includes all the outcomes achievable by that tissue **in response** to the environments present in the embryo at that particular stage.) Inductive interactions may be more or less complicated: only the induced tissue may change or both tissues may change and participate, as in morphogenesis, or, more commonly, several reciprocal inductive interactions may be required over a prolonged period of developmental time before a specific organ or tissue will form.

Types of Interaction

Two types of cell and tissue interaction have been defined by Holtzer: *permissive* and *instructive*.

In a *permissive interaction*, a signal from an apposing tissue is necessary for the successful self-differentiation of the responding tissue. This means a cell population (or the matrix molecules secreted by them) will maintain mitotic activity in an adjacent cell population. Since a variety of **different** cell populations may permit a **specific** cell population to undergo mitosis and cell differentiation, no specific instruction or signal, which may limit the developmental options of the responding tissue, is involved. Thus this signal does not influence the developmental pathway selected; there is no restriction. The responding tissue has the intrinsic capacity to develop and only needs appropriate environmental conditions to express this capacity. Permissive interactions often occur later in development, where a tissue whose fate has already been determined is maintained and stabilized by another.

An *instructive (directive) interaction* (induction) changes the cell type of the responding tissue (i.e. the cell population becomes restricted). Wessells (1977 [3]) proposed four general principles in most instructive interactions:

- (1) In the presence of tissue A, responding tissue B develops in a certain way.

- (2) In the absence of tissue A, responding tissue B does not develop in that way.
- (3) In the absence of tissue A, but in the presence of tissue C, tissue B does not develop in that way.
- (4) In the presence of tissue A, a tissue D, which would normally develop differently, is changed to develop like B.

An example of (4) above is the experimental association of chicken flank ectoderm with mouse mammary mesenchyme, resulting in the morphogenesis of mammary gland-like structures: chickens do not normally develop mammary glands (Sakakura 1983^[4]).

In an experimental context, instructive interactions have thus been more narrowly defined to describe the situation where the apposition of two dissimilar tissue types, which would not normally come in contact, results in one or other of those developing a unique morphology and gene expression, which would not normally be present (Sharpe & Ferguson 1988^[4]).

For any specific interaction, five basic questions can be asked:

- What is the tissue source for the inductive signal (i.e. where does the specificity reside)?
- What are the physicochemical properties of this signal?
- What is the mode of intercellular transmission?
- How do the responding cells process these signals?
- How do these signals result in the terminal differentiation of the cells and/or the morphogenesis of the tissue?

Many early experiments described the tissue source for the inductive signal, the responding tissue and the resultant morphogenetic effect. Recent advances have identified the nature of many of the signals and analogous substances which have similar effects, the variety of cellular transmission of the signals and the receptors and the consequences of signalling to the target tissue.

As will also be evident, however, in the subsequent sections, at a mechanistic level it is unclear whether there are fundamental differences between instructive and permissive interactions: they are more likely easily identifiable experimental outcomes, depending upon the nature of the signalling molecule, its divergent effects on the two tissues and the state of competence of the responding tissue. Tissue interactions continue into adult life and are probably responsible for maintaining the functional heterogeneity of adult tissues and organs. For example, there is complex tissue heterogeneity with sharply compartmentalized boundaries in the oral cavity; the distinction between the attached gingiva of the gums, the alveolar mucosa of the floor of the mouth and the lips, the vermilion border and the skin, are sharp and distinct boundaries of specific epithelial and mesenchymal differentiation and are almost certainly maintained by

continuing epithelial/mesenchymal interactions in adult life (for review see Sharpe & Ferguson 1988^[1]). Perturbation of these interactions throughout the body may underlie a wide variety of adult diseases, including susceptibility to cancer and proliferative disorders.

Epithelial/mesenchymal Interactions

These are a specific subset of embryonic tissue interactions involving signalling between an epithelial tissue and a mesenchymal tissue. They are particularly common and important during embryonic development. They provide a mechanism for coordinating and fine tuning the development, for example mitotic rate, differentiative ability, etc, of the two tissues, which are key for successful morphogenesis. As with general tissue interactions, epithelial/mesenchymal interactions are often described as instructive or permissive.

Generally mesenchymal populations control the pattern of development, i.e. whether an arm or leg develops, or whether stomach or colon develops; however, epithelial cells often retain their original cytodifferentiation and may have an early profound effect on the underlying mesenchyme. Thus in a recombination of chick gut epithelium and mouse mesenchyme, or vice versa, whereas the patterning of the intestinal villi will be determined by the underlying mesenchyme, the epithelial cells will produce the enzymes associated with the relevant species, i.e. mouse epithelium will produce lactase and chick epithelium sucrase, regardless of the origin of the underlying mesenchyme (Haffen et al 1989^[2]). Further, within gut development there is the suggestion that the epithelium may first influence the mesenchyme thus conferring its morphogenetic properties; a similar effect may be seen in the early stages of formation of the base of the skull, where the neuroepithelium, by placing specific molecules in the basal lamina, can halt migration of subjacent mesenchymal cells and initiate their differentiation along a chondrogenic pathway (see p. 274^[3]). Thus there are temporally reciprocal interactions between the epithelium and mesenchyme, the epithelium signalling to the mesenchyme, which then in turn signals back to the epithelium, etc. Such sequential spatial and temporal reciprocal interactions have been termed *epigenetic cascades* (Hall 1992^[4]); they lead to the differentiation and morphogenesis of most tissues and organs (Sharpe & Ferguson 1988^[5]; Hall 1992^[4]). An example of a reciprocal tissue interaction can be seen in mammalian tooth development summarized in 3.8^[6]. Other examples of epithelial/ mesenchymal interactions are used throughout the section to illustrate the mechanisms in each system. (For an excellent text on interactions consult Wessells 1977^[7].)

Mechanisms of Signalling

In theory, there are four principle mechanisms by which cellular interactions may be signalled: *direct cell-cell contact*, *cell adhesion molecules* and their receptors, *extracellular matrix molecules* and their receptors, and *growth factors* and their receptors. Many of these mechanisms interact and it is likely that combinations of them are involved in development. 3.9^[8] illustrates diagrammatically some ways by which mesenchymal cells could signal to epithelial cells. An additional set of identical mechanisms could operate for epithelial to mesenchymal signalling. Clearly, these mechanisms would increase in complexity, for example by reciprocal interactions

or by the divergent effects of a single molecule on epithelial and mesenchymal cells.

Direct Cell–Cell Contact

Gap junctions appear to be important for communication and transfer of information between cells. Antibodies to gap junctional proteins, experimentally injected into early amphibian embryos, appear to disrupt aspects of neural induction, and in mammalian embryos disruption of gap junctions at the 8-blastomere stage prevents compaction from occurring. It is suggested that gap junctional communication is involved in patterning (see below). The intercellular passage of dye between cells via gap junctions can be monitored, and the distribution of gap junctions both spatially and temporally can be studied in embryos by antibodies to *connexins*, constitutive proteins of gap junctions. Dye coupling of cells has been recorded as cells of the paraxial mesenchyme form epithelial somites, and between neuroepithelial cells within rhombomeres (Martinez et al 1992^[1]), and connexins have been noted between cardiac myocytes (Fromaget et al 1992^[2]) and in the media of the outflow tract of the heart (Minkoff et al 1993^[3]).








Endogenous electrical fields are also thought to have a role in cell–cell communication. Such fields have been demonstrated in a range of amphibian embryos and in vertebrate embryos during primitive streak ingression (Jaffe & Stern 1979^[4]; Winkel & Nuccitelli 1989^[5]); experiments in which endogenous currents were shunted out of embryos resulted in tail defects (Hotary & Robinson 1992^[6]). Neuroepithelial cells have been shown to be electrically coupled regardless of their position relative to interrhombomeric boundaries.

Cell Adhesion Molecules (CAMs or Cadherins)

These families of molecules which mediate adhesion between cells have been identified and their spatial and temporal distribution localized in the early embryo. They are thus candidates for signalling cellular interactions. Some of the most extensively studied CAMs include the *neural cell adhesion molecule* (*N-CAM*), first identified in nerve cells, *liver cell adhesion molecule* (*L-CAM*), first identified in the liver, *cell–cell adhesion molecule* (*C-CAM*) (Edelman 1986^[7], 1988^[8]), and *substrate adhesion molecule* (*SAM*). Although Edelman (1988^[8]), in his morphoregulatory hypothesis of development, directly implicated the expression of CAMs and SAMs in embryonic induction, current evidence indicates that their expression may represent an early response of groups of cells to embryonic induction signals and that such expression can modify the behaviour of groups of induced cells. To this extent cell adhesion molecules may be much more important in morphogenetic events.

Other molecules found in the extracellular matrix (see below), for example fibronectin and laminin inter alia, can modulate cell adhesion by their degree of glycosylation. Self-assembly or cross-linking by matrix molecules may affect cell adhesiveness by increasing the availability of binding sites or by obscuring them (Adams & Watt 1993^[9]).

Extracellular Matrix Molecules and their Receptors

These are synthesized by both epithelial and mesenchymal cells. *Epithelial cells* produce a two-dimensional *basal lamina*. A variety of matrix molecules including *laminin*, *fibronectin*, *type IV collagen* and various *proteoglycans* are found in basal laminae. The particular molecules can vary during development according to spatial and temporal patterns resulting in changes in behaviour of the underlying mesenchymal cells (e.g. see development of skull, p. 271 ). *Mesenchymal cells* produce extracellular matrix molecules in three-dimensions. Those adjacent to an epithelial layer will contribute to it forming a *basement membrane* which secures the epithelial layer to the underlying tissue, whilst those cells deep within a mesenchymal population may synthesize *matrix molecules* (fibrillar or granular) to separate cells locally, open migration routes, or leave information within the matrix to act on cell populations passing at a later time (Nathan & Sporn 1991 ). Molecules of the extracellular matrix are complex; they include more than 19 individual types of *collagen* (some of which are capable of being individually spliced to give more than 100 variants (Mayne & Burgeson 1987 ), *proteoglycans* and *glycoproteins* (which come in a wide variety of forms, with and without binding proteins); Scott 1989 , 1993; Knudson & Knudson 1993 ; Erickson 1993b , and *elastic fibres* (Rosenbloom et al 1993 ). Of particular interest is *hyaluronic acid*, a glycosaminoglycan. Because of its vast capacity to bind water molecules it creates and structures the space between the mesenchymal cells, creating much of the overall shape of embryos. Experimental removal of hyaluronic acid prevents the formation of cell migration routes, removes the support for overlying epithelia, and disrupts the normal branching of glandular systems.

Fibronectin deposited extracellularly along a migration pathway will affect cells which touch it later, causing realignment of their intracellular actin filaments and thus their orientation; fibronectin induces cell migration. The receptors for extracellular matrix molecules like fibronectin and laminin were termed *integrins* because they integrate (via α and β subunits which span the cell membrane) extracellular proteins and intracellular cytoskeletal elements, allowing them to act together. The binding preference of integrins depends upon their combination of subunits and environmental conditions.

Mutations of the genes which code for extracellular matrix molecules give rise to a number of congenital disorders; for example mutations in type I collagen give rise to *osteogenesis imperfecta*; in type II collagen they produce disorders of cartilage; and in fibrillin they lead to Marfan's syndrome.

Although all molecules secreted into the spaces around the mesenchymal cells are technically extracellular matrix molecules, growth factors are distinguished from them. Growth factors (see below) and extracellular matrix molecules share numerous cooperative properties; indeed there are huge stores of growth factor proteins bound and sequestered to extracellular matrix molecules, particularly those of the basal laminae. Growth factors interact with extracellular matrix molecules in the following ways:

- Growth factors can bind to specific extracellular matrix molecules and remain active
- Specific extracellular matrix molecules bind and neutralize growth factors

- Extracellular matrix molecules have growth factor repeat sequences which may function as:–building blocks–sites of growth factor activity–cell migration attachment sites
- There is cooperative secretion of extracellular matrix molecules and growth factors
- Growth factors stimulate extracellular matrix molecule biosynthesis
- Growth factors stimulate extracellular matrix receptor expression
- Extracellular matrix molecules down regulate synthesis of growth factors and/or their receptors.

It is believed that growth factor activation may be a key in signalling epithelial/mesenchymal interactions. (For an extensive review of extracellular matrix molecules see Adams & Watt 1993 [1].)

Growth Factors and their Receptors

Large families of growth factors and their receptors have been identified at the molecular level and their spatial and temporal distributions have been mapped during embryogenesis. Examples of such families include the *epidermal growth factor family* (EGF), the *transforming growth factor beta family* (TGFβ) (which includes the bone morphogenetic proteins), the *fibroblast growth factor family* (FGF), the *insulin-like growth factor family* (IGFs), the *platelet derived growth factor family* (PDGF), etc. Growth factors can (despite their name) signal a wide variety of cellular effects, including stimulation or inhibition of growth, differentiation, migration, etc. (Sporn & Roberts 1990 [2]). Because each family of growth factors is so large, and because there are a similarly extensive number of receptors, the possible signalling combinations are considerable. Often, a single growth factor can bind with varying affinities to individual receptor family members. Some of these receptors are monogamous, recognizing only one isoform, whereas others are polygamous, recognizing all isoforms. The effects of any individual growth factor may therefore depend on which receptor isoform, or ratio of isoform receptors, are displayed on the cell surface. It is now clear that developmental information resides, not in any single molecule, but rather in the combination of molecules to which a cell is exposed. Thus, varying combinations of growth factors in varying concentrations can elicit quite different effects on similar cells (Jessell & Melton 1992 [3]).

Examples of the importance of growth factors in embryonic signalling are mentioned throughout the section in the context of the development of the individual systems where this is appropriate. Two examples are given here. Members of the FGF family and TGFβ family (principally *activins*) are involved in signalling mesoblast induction (Jessell & Melton 1992 [3]). For example, isolated amphibian blastocoele roofs (termed, animals caps) treated with activin form dorsomedial mesoderm structures whereas treatment with FGF results in ventral components; activin alone (in the absence of hypoblast) will induce primitive streak formation in the chick epiblast. Palatal differentiation into nasal pseudostratified ciliated columnar cells, oral stratified squamous cells or medial edge epithelial cell adhesion and migration by the underlying

mesenchyme appears to be signalled by a variety of growth factors, for example *transforming growth factor alpha* (TGF α) (Dixon et al 1991^[1]), TGF β 1, 2 and 3 (Brunet et al 1995^[2]), IGFII (Ferguson et al 1992^[3]).

Growth factors can be delivered to and act upon cells in a variety of fashions: *endocrine*, *autocrine*, *paracrine*, *intracrine*, *juxtacrine* and *matricrine* (3.10^[4]). Interestingly, many growth factors are secreted in a latent form, for example associated with a propeptide (latency associated peptide) in the case of TGF β (Miyazono et al 1993^[5]; Taipale et al 1994^[6]) or attached to a binding protein, in the case of IGFs. Activity of the growth factor is dependent on dissociation from the binding protein or the latency associated protein, and therefore post-translational mechanisms of growth factor activation represent a critical control point for growth factor activity. Such activation mechanisms may involve *specific proteases* and/or *conformational changes* in the latency associated complex, by binding, for example, to a different receptor (in the case of TGF β , binding of mannose-6-phosphate residues on the latency associated peptide, to the mannose-6-phosphate/IGFII receptor; Taipale et al 1994^[6]).

The specific activity of various growth factors may equally depend on stabilization of the growth factor at the receptor by an accessory molecule. A good example of this is the delivery and stabilization of the activity of FGF2 by heparin sulphate proteoglycan (Walker et al 1994^[7]). Such complexity in the signalling system means that for most developing organs, there is at present only a rudimentary knowledge of the specifics of which combinations of signals give rise to which kinds of differentiation.


The diverse range of growth factors and their receptors, and their importance in developmental processes has been discussed by Ferguson (1994^[8]). He argues that the numerous growth factors and their receptors interact to give a smooth temporal integration of developmental events. Thus one growth factor may alter the synthesis of another growth factor, or its receptor. If the signalling systems are both hierarchical and functionally redundant, with each developmental event having a window of operational viability that may be achieved by a principal mechanism with a number of parallel backup mechanisms, then, if there were a disturbance in any one system its effects would be minimized by a parallel backup system. Thus whilst one main mechanism for developmental processes might be sought, this may not necessarily be the way that evolution has optimized embryonic development. Survival of the embryo may be considered the driving force behind evolution, with generation of phenotypic variation also an important phylogenetic force. Ferguson suggests that both of these may be achieved by utilizing developmental mechanisms which are quite slack, with appropriate backups, or self-balancing programmes, which ensure smooth development of the embryo, its survival and the generation of variation.




Thus it seems that epithelial and mesenchymal cell populations can structure the space around them by secretion of particular matrix molecules or growth factors which can in turn organize the cells that contact them. The extracellular matrix is structured rather than random so that cell–matrix interactions and matrix–cell interactions control: the position of migration routes; whether cells migrate or not; whether cells begin differentiation or not. Matrix molecules thus

propagate developmental instructions from cell to cell forming a far-reaching four- (spatial and temporal) dimensional communication mechanism.

Morphogenesis and Pattern Formation

Morphogenesis may be described as the assumption of form by the whole, or part, of a developing embryo. As a term it is used to denote the movement of cell populations and the changing shape of an embryo particularly during early development. The most obvious examples of morphogenesis are the large migrations occurring during gastrulation; however, local examples include *branching morphogenesis*.

The development of branches from a tubular duct occur over a period of time. In this case an interaction between the proliferating epithelium of the duct and its surrounding mesenchyme and extracellular matrix results in a series of clefts which produce a characteristic branching pattern (3.11 ). Normally during tubular and acinar development hyaluronidase secreted by the underlying mesenchymal cells breaks down the basal lamina produced by the epithelial cells and this locally increases epithelial mitoses forming an expanding acinus. The mesenchyme then initiates cleft formation by producing collagen III fibrils within the putative clefts. (If the collagen is removed no clefts develop; if excess collagen is not removed, supernumerary clefts appear.) The collagen acts to protect the basal lamina from the effects of the hyaluronidase and thus the overlying epithelia have a locally reduced mitotic rate. The region of rapid mitoses at the tip of the acinus is thus split into two and two branches develop from this point. This mechanism of branching morphogenesis is seen throughout the systems, from lungs to kidney and including most glandular organs.

Pattern formation concerns the processes whereby the individual members of a mass of cells, initially apparently homogeneous, follow a number of **different** avenues of differentiation precisely related to each other in an orderly manner in space and time. The 'patterns' embraced by the term apply not only to regions of regular geometrical order, for example the crystalline lens, but also to asymmetrical structures such as the tetrapod limb. For such a process to occur individual cells must be informed of their position within the embryo and utilize that information for appropriate differentiation; thus *positional information* is a fundamental concept for explaining mechanisms of pattern formation (Wolpert 1989 ). The picturesque model adopted to present this hypothesis is the so-called 'French Flag Problem' (3.12 ). In this a line of communicating cells are considered to have three possibilities for differentiation: blue, white or red, and they form a correctly proportioned French Flag whatever the number of cells in the line and even if parts of the original line are removed. It is assumed that each cell is assigned a positional value by appropriate signals with respect to boundaries at the ends of the line (3.12 ). Such a hypothesis does not only explain proportionate differentiation, but also epimorphic and morphallactic regeneration (see above). Thus positional information provides a unifying concept for understanding the development and regulation of a variety of patterns. The same signals and positional values may be used to specify different patterns; the differences arising from both developmental history and/or genetic constitution.

The essential features of a co-ordinate system to establish positional information are, *boundaries* with respect to which position is specified; a *scalar* which gives a measure of distance from the boundary; and *polarity* which specifies the direction in which position is measured from the boundary (Wolpert 1989). For a one-dimensional system, all the necessary features can be provided by a monotonic decrease in the concentration of a chemical (a morphogen), which could be set up with a localized source or by reaction diffusion. The concentration of chemical at any point then provides a scale or a measure of distance from the boundary and the slope of the concentration gradient effectively provides the polarity. Nearly all positional fields are small, none being longer than about 1 mm in maximum linear dimension, or about 50 cell diameters (most are much smaller). The time required to specify position appears to be in the order of hours (Wolpert 1989).

The term *morphogen*, first used by Turing in 1952, was originally used in relation to pattern formation; the distribution of a morphogen reflected the resulting overt pattern. Its use has now been extended to include a concentration gradient that specifies position (as above), i.e. a *positional* signal (Wolpert 1989). This definition clearly distinguishes between a positional signal (morphogen) and an induction signal (see above) as the latter does not specify pattern. Differences between these types of signal (as discussed by Wolpert 1989) are as follows:

<i>Positional signal</i>	<i>Inductive signal</i>
Involves same or different tissues	Interaction between two different tissues
Specification of multiple cell states	Specification of one cell state
Graded response	All or none response
Larger range signal	Shorter range signal
Polarity	No Polarity
Provided by or linked to boundary region	Relation to boundaries variable
Instructive	Instructive or permissive

The best example of a morphogen is the graded distribution of the protein encoded by the *bicoid* gene, which is the key regulator for patterning along the cephalocaudal axis of the *Drosophila* embryo (Nusslein-Volhard 1991; Lawrence 1992). The *gooseoid* gene (named because of its similarities to the *Drosophila* gooseberry and bicoid genes) serves the same function in vertebrate embryos (De Robertis et al 1992), and has been demonstrated in *Xenopus* and mouse (see p. 143). In vertebrates, *retinoids* (vitamin A derivatives) represent a major class of non-peptide growth factor signals that best fulfil the criteria for morphogens (Jessell & Melton 1992). In development of the limb (see p. 291) transplantation of the zone of polarizing activity from the postaxial border of the limb bud to the preaxial border results in mirror image skeletal duplication. This effect can be mimicked by applying retinoic acid to the preaxial mesenchyme (Tickle et al 1982), leading to the hypothesis that retinoic acid is the *endogenous polarizing signal* in the limb. It has been suggested that it acts by stimulating the local synthesis of TGF β family members particularly BMP2.

The Molecular Control of Embryonic Morphology

Two related themes have emerged recently that have revolutionized our understanding of developmental processes: (1) that the control of embryonic morphology has been highly conserved in evolution between vertebrates and invertebrates; (2) that this control involves families of genes coding for proteins that act as transcriptional regulators.

Homeobox

The fruit fly *Drosophila* possesses eight *homeotic genes* which specify the structures developing on each body segment. There are two regions on *Drosophila* chromosome 3 that contain these genes: one region, the *Antennapedia complex (ANT-C)*, contains five genes; the second, *Bithorax complex (BX-C)*, contains three. The existence of these genes was determined by the study of *homeotic mutations* which result in conversion of one body part of the fly to another, for example legs developing on the head in place of antennae. The mutations involved single genes which, when cloned, all had a highly conserved sequence of 183 base pairs (bp), coding for 61 amino acids at the C-terminus of the protein. This sequence was termed the *homeobox*. Investigation of its structure revealed that it formed a region in the protein that bound to DNA (reviewed by Scott 1989^[1]). One of the intriguing features of the homeotic genes is that the linear order of each gene, from the 3' end to the 5' end, is the same as its expression, along the cephalocaudal axis of the embryo, a feature referred to as *collinearity*. Further analysis of genes involved in the control of segmentation in the fly showed that many of them also contain homeobox sequences but with little else in common. Thus, many of the genes known from mutations to be essential for normal embryonic morphology had one small region in common (the homeobox) that codes for a DNA-binding function. This suggests that embryonic morphology in *Drosophila* is controlled overall by DNA-binding proteins that function as transcriptional regulators, controlling the expression of other genes. These other genes might consist of genes encoding structural proteins, growth factors, cell adhesion molecules, signalling molecules, cell surface proteins, extracellular matrix proteins, enzymes, etc. that act in concert to generate tissue morphogenesis.

Vertebrate Hox Genes

The identification of genes containing homeoboxes in vertebrates raised the possibility that such genes might have similar functions in vertebrate embryos as in invertebrate embryos, assuming that evolutionary conservation of the homeobox implies an important role. Currently, 38 genes containing homeoboxes are known in mammals, and all show a high degree of similarity with the homeoboxes of the *Drosophila* homeotic genes. These genes are called *Hox (murine)* or *HOX (human)* and are found in four *clusters* known as A (mouse chromosome 6, human chromosome 7), B (mouse chromosome 11, human chromosome 17), C (mouse chromosome 15, human chromosome 12), and D (mouse chromosome 2, human chromosome 2). The *Hox* genes are numbered from 1 to 13, with 1 corresponding to a cephalic gene and 13 a more caudally placed gene. *Hox a-6*, *Hox b-6*, *Hox c-6* and *Hox d-6* are located at the same relative positions in their respective clusters and are referred to as a *paralogous group* (3.13^[2]) (Scott 1992^[3]).

It should be noted that an earlier nomenclature of *Hox* genes reflected their order of discovery

rather than any logical system. The nomenclature described above was recommended by the Mouse and Human Gene Mapping Nomenclature Committees (Scott 1992^[4]). It provides a system that identifies all the *Hox* genes in their relative positions and allows some flexibility for the discovery of genes in other vertebrates. The Nomenclature Committee also noted that the term *Hox* should be used only for vertebrate genes related to the *Drosophila* *ANT-C* and *BX-C* gene clusters, by position in a complex, and by their sequence and expression along the cephalocaudal axis. Sequence similarity alone would not qualify for the use of the *Hox* name. Thus genes previously termed *Hox-7* and *Hox-8* are renamed *Ms x-1* and *Ms x-2* respectively.

All *Hox* genes have a number of features in common: they all have homeoboxes at their 3' ends that are related to *Drosophila* homeotic gene homeoboxes; they all have a single small intron of around 1 kb with the homeobox being in the second exon; they are all transcribed in the same direction; and they are all expressed in the embryonic nervous system, somites and limbs.

The outstanding feature of the *Hox* genes is their remarkable *conservation* with the fly homeotic genes. Based on amino acid sequence and cluster position, each paralogous group of *Hox* genes can be traced to a *Drosophila* homeotic gene. Thus, the *Hox* genes are *phylogenetically* related to the homeotic genes and have arisen via duplication of an original ancestral invertebrate cluster. Even more remarkable is that the collinearity of homeotic gene position and embryonic expression is also the same for *Hox* genes. Thus, the closer to the 3' end a gene is in a cluster, the more cephalic is its expression in the embryo (3.13^[5]). Paralogous genes, being related to the same ancestral gene, show similar cephalic expression domains, but interestingly have different dorsoventral domains in the developing CNS.

The level of evolutionary conservation between homeotic and *Hox* genes implies that they have similar functions and thus *Hox* genes might function to control morphology in mammalian embryogenesis. Direct manipulation of *Hox* gene expression in murine embryos has shown this to be the case (see below).

Hox Codes

Hox genes are expressed in developing ectodermal structures, such as the rhombomeres and neural crest, in a wide variety of mesenchymally derived organs such as the somites, heart, kidney, testis, etc. but not in any endodermally derived structures. The striking feature of *Hox* expression is that the genes have overlapping expression domains but with very distinct cephalic boundaries. Thus, at any point along the cephalocaudal axis of the embryo, cells of the CNS and paraxial mesenchyme (which later forms somites) have a characteristic complement of *Hox* gene expression. This complement of *Hox* genes (homeoproteins) is believed to form an *axial code* (*Hox code*) that specifies position along the cephalocaudal axis.

Hox codes have been identified in four different locations in the embryo:

- an *axial code*, specifying somites (Kessel & Gruss 1991^[6])
- a *branchial code* specifying neuronal and neural crest development in the branchial

region (Hunt & Krumlauf 1991; Hunt et al 1991a).

- an *organogenesis code* (a variation of the axial code) (Gaunt et al 1988).
- a *limb code* (Dollé et al 1989).

In all these regions, the overlapping domains of *Hox* gene expression have been interpreted as providing specific positional information (3.14). For example, in the hindbrain the cranial boundaries of expression of *Hox* genes correspond to the morphological boundaries, the rhombomeres (3.14A). The expression of *Hox* genes in the rhombomeric neuroepithelium is believed to specify the rhombomeres and pattern the resulting motor neurons. This *Hox* gene expression is also carried over into the cranial neural crest cells according to their position of origin, imparting upon them positional information which will be used to determine their ultimate fates (3.14B; Hunt et al 1991b). (For further description of *Hox* gene expression in the rhombomeres see p. 228.)




The importance of the axial code in specification of somites is suggested by disruption of the *Hox c-8* gene which results in a transformation of the first lumbar vertebra into an extra thoracic vertebra (T14) complete with ribs (Le Mouellic et al 1992). In developing avian limb buds, ectopic expression of *Hox d-11* results in disruption of the digit pattern (Morgan et al 1992). This is most evident in the leg when *Hox d-11* is ectopically expressed in more preaxial regions resulting in a preaxial toe that looks like the adjacent index toe (Morgan et al 1992).

Retinoic Acid and *Hox* Codes










The teratogenic effects of retinoic acid (a derivative of vitamin A) have been extensively studied for over 25 years. Embryos (human and rodent) exposed to excess retinoic acid show abnormalities of many different organs, the most striking being craniofacial and limb defects (reviewed by Morris-Kay 1993). The most intriguing feature of craniofacial retinoic acid teratogenesis is that the abnormalities produced appear to originate from abnormal development of the hindbrain. This, together with the fact that *Hox* gene expression in cultures of embryonic carcinoma cells is sensitive to exogenous retinoic acid concentration, led to the conclusion that retinoic acid exerts its teratogenic effects on craniofacial development by particularly altering *Hox* gene expression (Simeone et al 1990). Retinoic acid induces a transformation of rhombomeres 2/3 into a 4/5 identity, with a corresponding transformation of the trigeminal motor cranial nerve into a facial motor cranial nerve (Marshall et al 1992). Neural crest cells derived from rhombomeres 2/3 also appear caudalized since they express *Hox* genes corresponding to 4/5.

Similar effects of excess retinoic acid on vertebral and limb development are also consistent with its affecting *Hox* gene expression and disrupting the *Hox* code. Caudal transformations of vertebrae along the complete body axis observed with retinoic acid administration are postulated to result from alteration of *Hox* gene expression (Kessel & Gruss 1991).










The limb *Hox* code with respect to the digit pattern consists of overlapping domains of the

caudal *Hox d* genes (*Hox d-9–d-13*) (see p. 293  and 3.14B ). Implantation of retinoic acid soaked beads into the preaxial margin of the developing limb results in a mirror-image duplication of the digit pattern (see above) and duplication, in a mirror fashion, of the expression of *Hox d* genes consistent with their role in determining the digit pattern (Izpisua-Belmonte et al 1991 .

Non-Hox Homeobox Genes

It is now clear that the *Hox* genes represent only one particular group of genes having homeoboxes and that there are many more homeobox genes that are **not** clustered and which are only related to *Hox* genes by virtue of having a homeobox. These homeobox genes are called by a variety of names, for example *Msx*, *Mox*, *Dlx*, *Otx*, *Cdx*, *Emx*, *Goosecoid*, etc.; they have widely varied expression patterns in embryos. The common feature of these genes is that on the whole their expression is unrelated to the cephalocaudal expression of the *Hox* genes and they are often highly restricted to particular cells in developing organs. Whether any of these genes produce 'codes' for development of individual organs remains to be seen. Many of these genes, in common with *Hox* genes, are related to individual *Drosophila* homeobox genes but in vertebrates they are duplicated to varying extents. Thus, *Drosophila* has a single muscle specific homeobox (*msh*) gene, whereas mammals have three *msh*-related genes, *Msx-1*, *Msx-2* and *Msx-3* (Hill et al 1989 ; Robert et al 1989 ; Holland 1991 ; MacKenzie et al 1991 , 1992 ). Similarly, the *Drosophila distalless* gene has at least five related genes (*Dlx-1–5*) in mammals (Cohen et al 1989 ; Price et al 1991 ; Porteus et al 1991 ; Robinson et al 1991 ). It is probable that duplication of these genes occurred progressively during evolution of more complex body plans again demonstrating a link between morphology and homeobox genes.

Pax Genes

Pax genes belong to a family of morphogenetic regulatory genes, initially identified by homology to the *Drosophila* segmentation gene *paired'*, which encodes DNA binding transcription factors (reviewed by Hastie 1991 ; Gruss & Walther 1992 ). A total of 9 genes have so far been identified containing this *paired'-box* (a sequence seen in the *paired'* gene), and four of this group also contain a homeobox (Walther et al 1991 ; Stapleton et al 1993 ). *PAX* genes have proved to be particularly interesting, largely because at least two of them, *PAX-3* and *PAX-6*, have been shown to be the genes that are mutated in two human genetic diseases, Waardenburg's syndrome (Baldwin et al 1992 ; Tassabehji et al 1992 ) and aniridia (Ton et al 1991 ; Jordan et al 1992 ) respectively. Waardenburg's syndrome is responsible for 2–3% of total congenital deafness and accompanying features include dystopic canthorum and pigmentary disturbances such as frontal white blaze of hair (a disruption of neural crest cell development). The aniridia phenotype has a failure of iris development and abnormal retinal development. Both these conditions are autosomal dominant. Both syndromes have equivalent mutations in mice, namely *Splotch* (Waardenburg) and *Small eye* (aniridia) caused by mutations in the *Pax-3* (Epstein et al 1991 ) and *Pax-6* genes respectively. In common with the transgenic mutations in *Hox* genes, the *Pax* mutations only produce abnormalities in a *subset* of tissues where they are expressed. Thus, although both genes are expressed in the developing CNS, no major

abnormalities are seen here in the mutants.

Transgenics, Gene Knock-Outs and Functional Redundancy

A method for investigating the function of a particular gene during development is to knock out the gene using deletional mutations in embryonic stem cells, followed by the production of embryonic chimeras and subsequently transgenic animals carrying the null phenotype. A variation on such an approach is to construct a transgenic animal in which manipulation of the promoter region of the gene results in expression either in the wrong place and/or at the wrong time. As integration of exogenous genetic constructs within the genome is somewhat random, occasionally the construct inserts at a position where it disrupts an important host gene, thus creating an insertional mutant. In such mutants the inserted DNA acts as a marker so that subsequent cloning of the region surrounding it reveals the important host gene, for example in the case of x-linked cleft palate (Wilson et al 1993^[4]).


The function of a developmental gene can also be inferred from the distribution of its protein (as revealed by immunolocalization) or messenger RNA (as revealed by in situ hybridization) or by functional studies (such as addition or deletion of the gene product). In several instances, targeted disruption of a gene using transgenic knock-out approaches produces a phenotype similar to the one predicted from such expression studies (Satokata & Maas 1994^[4]). Other targeted inactivations result in early embryonic death, as clearly the gene product is required for early embryonic development: this can often be avoided by expressing the transgene from a tissue-specific promoter, which is active later in development (Werner et al 1993^[4]). However, one astonishing theme appears to be emerging, namely that the disruption of a supposedly important gene frequently produces a minimal or null phenotype, and apparently normal animals are born (Erickson 1993b^[4]; Ferguson 1994^[4]).

Generation of the Embryonic Body Plan



Specification of the Body Axes

Embryos may be considered to be constructed with three orthogonal spatial axes, plus a further temporal axis. In anamniotes the mechanisms by which the axes of the embryo are established are to some extent understood. Dorsoventral and cephalocaudal axes are specified by changes in the zygote cytoplasm at fertilization. In mammalian embryos axes cannot be specified at such early stages and it is only after the early extraembryonic structures have been formed and the inner cell mass can be seen that *embryonic axes* can be defined.

In amniotes, the *dorsoventral axis*, described as the first axis (Gurdon 1992^[4]), has been identified by the appearance of the epiblast. In reptilian and avian embryos the blastodisc layer of cells separates into two, the upper layer of which forms the epiblast. In mammals the future epiblast can be predicted when the hollow blastocyst has formed. The inner cell mass becomes

(seemingly) randomly located on the inside of the trophoctoderm and forms a population of epiblast cells subjacent to the trophoblast. This region implants first. It is not known whether the trophoctoderm in contact with the inner cell mass initiates implantation so that the future dorsal surface of the embryo is closest to the disrupted maternal vessels at the implantation site, or whether the inner cell mass can travel around the inside of the trophoblast to gain a position subjacent to the implantation site once implantation has commenced; the latter has been suggested as likely (O'Rahilly & Muller 1987.

It is worth reflecting at this point that the whole **initially flat** embryonic disc may be conferred with axes; however, their subsequent orientation in the folded embryo, at the body plan stage, will be utterly different. Only a circumscribed central ellipse of the early embryonic disc will form dorsal structures in the folded embryo; the remainder of the disc, to its periphery, will form lateral and ventral structures. In mammalian embryos the peripheral edge of the disc will become constricted at the umbilicus. Thus, although the appearance of part of the epiblast is noted as the specification of the dorsal surface of the embryo, the inner layer, i.e. the hypoblast, is not by default a ventral embryonic structure.

It is not until the appearance of the primitive streak that a true primary axis is conferred on the embryo, the *cephalocaudal axis*. The underlying hypoblast cells, which do not contribute to the embryo proper, induce the streak formation **and** its orientation (see p. 142). If the hypoblast at the caudal region of the embryonic disc is rotated with respect to the overlying epiblast a new streak will form according to the new orientation of the hypoblast (Azar & Eyal-Giladi 1981.

At gastrulation ingression of epiblast through the primitive streak produces a population of mesoblast cells which, by the position through which they ingress, have axial and medial or lateral characteristics assigned to them. Cells ingressing through the primitive node will give rise to (axial) notochord cells and cells of the medial halves of the somites (as well as embryonic endoderm), whereas the lateral halves of the somites and the lateral plate mesenchyme come from epiblast populations either more caudally placed in the primitive streak or ingressing later. The axial and medial populations will remain as **dorsal** structures in the folded embryo, and the surface ectoderm above them will exhibit dorsal characteristics; the lateral plate mesenchyme will become **lateral** and **ventral** after embryonic folding, and the surface ectoderm above this population will gain ventral characteristics. Dorsoventral specification of the developing limbs appears to reside in the surface ectoderm, and may be so designated at this early stage. Thus the dorsoventral axis of the *folded embryo* is also specified by the passage of epiblast cells through the primitive streak.

Presumptive head structures and prospective dorsal mesoblast are located close to each other immediately adjacent to the primitive node, whereas cells destined to be caudal and ventral arise from the lateral epiblast and converge to the posterior end of the streak. However, the mesoblast has the ability to be regionalized into different dorsoventral cell types until the beginning of neurulation.

The third and last spatial axis is the *bilateral*, or *latero-lateral axis*. This is present as a




consequence of the development of the former two axes. Initially the right and left halves of the embryonic body are bilaterally symmetric and in two places on each side of the body wall (somatopleure) lateral projections, the upper and lower limbs, develop.

With the last axis in play, the temporal, modification of the embryo relative to its original axes can be seen. The segmental arrangement of the cephalocaudal axis is very obvious in the early embryo and remains in many structures in adult life; so too dorsal embryonic structures remain dorsal and undergo relatively little change. The originally midline, ventral structures, however, especially those derived from splanchnopleuric mesenchyme, such as the cardiovascular system and the gut, are subject to extensive shifts, changing from a bilaterally symmetric arrangement to a whole body that is now chiral (see below).


Segmentation in the Embryo

The identification of *Hox* genes which control the development of segments of *Drosophila* embryos in vertebrate embryos provides a framework to explain the segmental structures which develop during ontogeny, many of which are to be replaced by derived non-segmental arrangements later.

The grouping of vertebrates as a separate subphylum of chordata is based on the possession of a *segmented vertebral column*. (Repeating body segments of this nature are termed *metameric* implying a repetition of basically similar structures.) Evidence suggests that the developmental processes involved in forming vertebrae may be responsible for many of the other segmentally arranged tissues and systems including the peripheral nervous system (PNS), the cardiovascular system, and, to a limited extent, the patterning of skin appendages.

Vertebrae are formed from bilateral aggregations of mesenchymal cells derived from ingression of the epiblast at gastrulation (see pp. 100 , 142 ). The cells migrate to lie lateral to the notochord where they are termed *paraxial mesenchyme*. As the embryo begins neurulation the paraxial mesenchyme segments to form discrete populations of cells termed *somites*. Cells ingressing through the lateral edges of the primitive node form the medial halves of the somite whereas cells ingressing through the cranial portion of the primitive streak form the lateral halves (see p. 143 ). The mesenchymal population of each somite undergoes a transformation to epithelium, with the cells forming gap junctions. Later the original medial portion of the epithelial somite reverts to mesenchyme to become the *sclerotome*, while the lateral part remains as the *epithelial plate of the somite* (also termed *dermomyotome*) which acts as a germinal epithelium for myogenic cells collectively termed the *myotome*. The cephalocaudal region of the embryo corresponding to individual somites is defined as the *fundamental metameric segment*, and thus the initial myotomes are segmental.


Sclerotomal cells migrate medially to surround the notochord. At this time differences are apparent between the cranial and caudal halves of the sclerotome. The cranial portion has binding sites for peanut lectin, cytotoxin and tenascin. Individual vertebrae form from a fusion



of the caudal half of one somite and the cranial half of the somite below (see also p. 266 ). The intersomitic cleft which thus develops (fissure of von Ebner) is a boundary zone. The reorganization of the sclerotomes along these lines results in the myotome of each somite spanning two adjacent vertebrae, i.e. each *segmental* somite contributes to cranial and caudal halves of two adjacent *intersegmental* vertebrae.

The myotomes generally produce two main muscular blocks, the *epaxial muscle group* which forms sequentially arranged erector spinae muscles, and the *hypaxial muscle group* which merges into a muscle mass to form the trunk muscles of the anterolateral body wall. The limbs and tongue muscles are formed by local myogenic populations arising from the ventrolateral edge of specific somites.

Motor neurons grow out from the CNS, and, with sensory axons from the neural crest cells of the dorsal root ganglia and autonomic axons, they *preferentially* migrate through the cranial portion of each sclerotome. Thus, in higher vertebrates, the subdivision of the sclerotome is responsible for generating the segmental arrangement of the PNS and ensures the exit of the spinal nerves between the vertebrae.

Each primitive dorsal aorta gives rise to paired, *ventral segmental arteries* which supply the gut, *lateral segmental arteries* to the mesonephros and bilateral *intersegmental arteries* to the body wall. These latter vessels persist almost unchanged in the thoracic and lumbar regions as the intercostal, subcostal and lumbar arteries. The venous drainage of the body is into intersegmental veins and the azygos system.

Characteristically a somite was described as giving rise to a segmental strip of dermis supplied by a sensory spinal nerve, thus giving rise to the term *dermatome* as used in medical practice. Another use of the term, to refer to a portion of a somite, is no longer used with an analogous meaning. The epithelial plate of the somite produces mainly myogenic cells which give rise to all of the voluntary muscle of the trunk and limbs. Some cells arise from the somites which contribute to the connective tissue of the dermis superjacent to the epaxial muscles and result in the patterning of the overlying epidermis. Patterning of the epidermis of the ventral part of the trunk and of the limbs is controlled by the somatopleuric mesenchyme (see p. 291 .

The nephrogenic mesenchyme (see p. 199 ), which develops slightly later between the somites and the coelom, has been regarded as being segmentally influenced. The pronephros of lower vertebrates develops nephrostomes and nephrocoeles (characteristically joined to the somite lumen in some cases). However, more recent studies have shown that, apart from their segmental blood supply, the functional pronephroi and mesonephroi of reptilian embryos do not appear segmentally organized (Collins 1990 ) (mammalian embryos do not possess a functional pronephros).

Within the head, segmentation is also seen relative to the branchiomic arches. Here the *Hox* genes have an overlapping expression of genes which coincide with the rhombomeric boundaries in the hindbrain. Specification of the neural tube, branchiomic motor and sensory nerves, and

neural crest mesenchyme appears to be controlled by the *Hox* genes, resulting in the typical arrangement of cartilage, nerve and blood vessels seen in each arch. Patterning of the arches is controlled by the neural crest mesenchyme.

Segmentation in the head and expression of the *Hox* genes is noted rostrally only as far as the rostral end of the paraxial mesenchyme and the notochord. Structures rostral to the notochord have been termed part of the prechordal or new skull; they are extensively derived from neural crest. The most rostral portions of the brain, however, have been shown to express a range of other genes (not associated with the *Hox* codes; see p. 255^[1]); generally they seem to have a nested arrangement similar to gene expression in the limb. Other studies have demonstrated a banding pattern of lineage within the cortex, with cells respecting a line between cortical and basal forebrain (see p. 255^[2]).

Thus the basic body plan of the embryo is drawn to segmental grid lines with boundaries between the segments maintained by different expression of genes and proteins, etc. which restrict cell migration in these regions. Organogenetic processes modify this initially segmental vertebrate body plan. The development of many systems illustrates either a retention of a segmental plan (e.g. spinal nerves, see p. 226^[3]) or its local replacement (e.g. the modifications of somatic intersegmental vessels by the development of longitudinal anastomoses, see p. 318^[4]). Abnormalities may result from improper specification of segments along the cephalocaudal axis as well as failure to produce the appropriately modified segmental plan.

Lateralization in the Embryo

Externally the body appears symmetric about the midline but the viscera are *asymmetric* such that the body plan is not superimposable upon its mirror image. It is a puzzle that this asymmetry is *handed* (chiral) rather than random, that is, it is almost always asymmetric in the same direction. For example, the cardiac apex is directed towards the left and the liver lies to the right. When the structure or arrangement of one or more of the laterally asymmetric organs is abnormal it is thought that errors in the specification or interpretation of left–right information may have occurred in early embryogenesis. Defects of lateralization may be responsible for some congenital anomalies of the heart, bronchial branching, lung lobation, major vessels, portal venous anatomy, the spleen and gastrointestinal mesenteric attachments. The frequent association of these anomalies with one another strengthens the view that they are due to a common defect and that this defect is one of lateralization. However, there are other defects also commonly seen with these abnormalities whose association requires a different explanation since the pathology seems to be independent of positional information. For example, pathology is seen in the lungs, ears and sinuses in *primary ciliary dyskinesia* and in the kidneys, liver and pancreas in cases of *renal-hepatic-pancreatic-dysplasia* when associated with abnormal situs (Lurie et al 1991^[5]). Another non-lateralized association is agnathia (Pauli et al 1981^[6]), which may be due to an insult occurring at the stage when both sidedness is specified and the mandible first forms.

In primary ciliary dyskinesia, the clinical manifestations of recurrent chest infection, sinusitis

and male infertility are well explained by the ciliary and flagella dyskinesia (Greenstone et al 1988^[5]), but the link with abnormal situs, which appears random, is not. Afzelius (1976^[6]) suggested that orientated embryonic cilia may establish visceral handedness and cells with single cilia have been noted in embryonic tissues including the murine primitive node and early notochord (Fujimoto & Yanagisawa 1983^[7]), but these embryonic cilia are likely to be immotile. Primary ciliary dyskinesia includes a heterogeneous collection of autosomal recessive ciliary dyskinesias in which most commonly there is a defect in *dynein*, a ciliary and intracellular motor protein. This has been suggested as a candidate component in the establishment of lateralization (Brown et al 1991^[8]) but sometimes in primary ciliary dyskinesia the dynein is normal yet there may still be situs inversus. Alternatively since the orientation of cilia in this condition shows greater variance than normal (Rautiainen et al 1990^[9]) it may be that a common mechanism is responsible for both the orientation of laterally asymmetric information and also for ciliary orientation.

Renal-hepatic-pancreatic dysplasia is sometimes associated with abnormal situs and it may be that two closely linked genes, one perhaps associated with tubule formation and another associated with the specification of situs, have both been disrupted. Whatever the mechanism, the association of these defects with abnormal situs provides clues to the developmental biology and cautions for the paediatrician and surgeon.

Abnormal Situs

In amniotes, the organs develop from a bilaterally symmetric epiblast. The first overt morphological asymmetry is a greater convexity of the right border of the midline embryonic heart tube (Patten 1922^[10]). This precedes looping, also towards the right, which places the ventricles and vessels in the correct arrangement for later function and septation. That asymmetry is first apparent in the heart may be no more than a reflection that the heart is an early organ to develop from a previously covert asymmetry; however, abnormal lateralization of the cardiac segments has unquestionably serious consequences and abnormal situs may occur in more than one segment of the heart tube. For example, there can be mirror-image dextrocardia in which all segments are inverted, or transposition of the major vessels alone, or congenitally corrected transposition, or atrial isomerism. Clinically, the site of the atrial appendages provides the best guide to the associated cardiac and vascular anomalies (Anderson et al 1990^[11]), from which the prognosis is best predicted.

Dextral looping of the embryonic heart tube is highly conserved in vertebrates, but the mechanism of looping and its consistent handedness are not understood. Conservation of the direction of looping across the phyla suggests that similar genes and mechanisms may be involved.

A normal arrangement of the cardiac atria, ventricles, vessels and viscera is referred to as *situs solitus*. However, there is no agreed terminology for patterns which include abnormally lateralized organs. The terms *situs inversus*, *situs inversus partialis*, *situs indeterminus*, *heterotaxy* and *asplenia/polysplenia* or *splenic syndromes* have been used, often without

definition. The abnormal arrangements are so varied and include almost all permutations of sidedness of the asymmetric organs that no single term provides a satisfactory description for any one case. An unpaired asymmetric organ such as the stomach may have one of three arrangements: *solitus* (the usual arrangement), *inversus* (mirror-image arrangement), or *ambiguous* (indeterminate). With paired organs that differ morphologically from their contralateral partner, such as atria, bronchi, lung lobes, arrangement may similarly be either usual or mirror image, but in addition, a situation of symmetry can occur. For example, there may be the same number of lung lobes on each side, or both atria may have the same sidedness, as assessed by the atrial appendages, either both left- or both right-sided in appearance. Such arrangements are called *isomerisms*.

When inversion or isomerism is found, the location of individual organs, vessels, cardiac segments and defects should be documented to provide a complete description in any one case, and uninformative terminology avoided.




Aetiology of Lateralization

There are both genetic and environmental influences in the aetiology of abnormal lateralization. Autosomal recessive (Arnold et al 1983), an X-linked pattern (Mathias et al 1987), and possible autosomal dominant pedigrees (Niikawa et al 1983) have been described. In animals various teratogens can cause situs inversus, sometimes alone (Fujinaga 1992) but more often with other anomalies also (Fujinaga & Baden 1991). In both humans and some strains of mice, defects of lateralization are seen amongst the congenital anomalies of offspring of diabetic pregnancies. A mitochondrial source of oxygen-free radicals during gastrulation has been suggested to play a role in this aetiology (Lander & Brown 1994).



In the mouse an autosomal recessive allele *inversus viscerum* (*iv*), causes situs inversus in just under half of the homozygotes, situs solitus with the same frequency and a few isomerisms and discordant inversions (Hummel & Chapman 1959). The spectrum of anomalies is very similar to the patterns of lateralized defects seen in man, including annular pancreas and preduodenal portal vein. The cardiac and splenic anomalies seen with the atrial isomerisms (Seo et al 1992) are also similar to human defects. The *iv* mutation lies near the immunoglobulin heavy-chain-constant-region complex (Igh-C) on chromosome 12 (syntectic to human chromosome 14q3) (Brueckner et al 1989; de Meeus et al 1992).

A second mutation *inv*, also recessive, has occurred in a transgenic mouse *OVE210* in which 100% situs inversus is reported in homozygous offspring (Yokoyama et al 1993). The flanking sequences map to chromosome 4. The defect is fatal in homozygotes before 7 days postnatal and dysplasia of the liver and renal tract contribute to death. It is not yet known if this model represents the renal-hepatic-pancreatic dysplasia seen in abnormal situs in humans. If it does, it is possible that two or more closely linked genes involved in situs and in tubule development have been disrupted by the transgene.


Cellular and Molecular Models of Lateralization

The body plan is handed, i.e. it is chiral, but as yet it is not known where *handed* information comes from or the nature of the mechanism of its expression. One model (3.15 ) suggests handedness to be signalled by an orientated chiral molecule fixed with respect to the craniocaudal and dorsoventral axes. Brown and Wolpert (1990 ) illustrated this with a hypothetical chiral 'F' molecule that could align its vertical limb with polar microtubules arranged in the craniocaudal direction and normally to be fixed in a frontal plane so that its arms point to a specific side. They depicted the 'F' molecule in cells that have been polarized with respect to the midline. Each cell could then establish the side of the embryo on which it is situated. This then establishes handedness at the level of the tissues. To account for situations of random handedness, as is seen in primary ciliary dyskinesia and in the *iv/iv* mouse, Brown and Wolpert proposed a means of generating a left–right gradient across the embryo at random. A homogeneous distribution of a biochemical factor across the midline could become asymmetric by amplification of thermal noise through a reaction-diffusion mechanism (Kauffman et al 1978 ). In normal circumstances this amplification could be biased by the handed cellular asymmetry. Organs and tissues then interpret the gradient and express their sidedness accordingly. Absence of the 'F' molecule leads to randomization of the left–right gradient and a 50% incidence of situs inversus.

The Brown-Wolpert model was elaborated to explain the observation that abnormal situs is frequently seen in the right conjoined twin. They hypothesized that once a left–right difference is established a property becomes fixed on the right, such that after bisection left-right cannot be re-established in the right twin but the labile left side can respecify to give normal situs in the left twin.

A mutation leading to reversed orientation of the 'F' molecule may account for the 100% situs inversus seen in *inv/inv* mice. However, other mechanisms for producing 100% inversion without reversing the primary handed information have been proposed (Brown & Lander 1993 ; see 3.16 ). Sequencing the genes for the normal alleles at *iv* and *inv* may illuminate steps in the establishment of situs but still may not tell us how it is specified.

Other Examples of Handedness


Other examples of handedness not linked with body plan handedness also occur in humans, for example handedness in cerebral dominance and hand preference. However, in these cases the incidence of left handedness in those with defects in situs is the same as in the general population. Animals are individually handed but this is random at the population level which may suggest that cerebral lateralization, important in the neurobiology of language, is quintessentially human (Corbalis 1989 ). Though not directly linked, similar mechanisms and homologous genes may be involved in the establishment of situs and cerebral lateralization.

That the organs are asymmetric may not be surprising but it is far from clear why or how they are asymmetric in the same direction, and why this should be so universal. It is clear that when lateralization is abnormal there are frequent cardiac, vascular, splenic and mesenteric anomalies













of clinical importance. Correct lateralization seems to be of crucial importance to survival.

Early Human Development

Female Gamete

The life cycle of the mammalian oöcyte starts in early embryos, when the primordial germ cells first differentiate, and is culminated many years later when a mature cell is fertilized. The development of germ cells into fertile oöcytes can be divided into several distinct phases, including: establishing the germ cell population; oöcyte growth and meiotic maturation; and growth and development of the antral follicle (3.17 ).

Establishing the Germ Cell Population

In mammals it is accepted that there is not a determined germ line as exists in drosophila and probably in frogs. The precise origin of the primordial germ cells in humans is not clear. In mice the germ cells are first recognized as a clump of about 40 alkaline positive cells in the extraembryonic mesoblast of 7.5 day old embryos (Ginsburg et al 1990 ). In humans of about 6 weeks postconception the germ cells migrate from their extragonadal site of origin to the gonadal ridges where they proliferate. By 8–10 weeks gestation about 600 000 oögonia populate the developing ovary (Baker 1963 ); from 12 weeks they begin their differentiation into primary oöcytes. The ovary is fully colonized in the fifth intrauterine month with maximal numbers reaching nearly 7 000 000 (Baker 1963 ). Primary oöcyte numbers then decrease so that by birth there are about 1 000 000 (4000 in mice) (Block 1953 ; Baker 1963 ; Baker 1972  (3.18 ). After birth a further degeneration occurs so that by puberty only 40 000 oöcytes remain (Pinkerton et al 1961  (3.18 )) and of these only 400 are ovulated during the reproductive lifespan of the female. This widespread degeneration is a feature of germ cell development in all mammals studied (Ingram 1962 ; Baker 1972 ) and appears to occur around pachytene, when crossing over takes place (see below). It has been suggested that meiotic pairing anomalies induce this atresia of fetal oöcytes (Speed 1988 .

From an early time the oöcytes become enclosed by somatic cells of the fetal ovary, surrounded by a basement membrane. This forms a unit known as a *primordial follicle*. The development of the primordial follicles is the final stage in establishing the germ cell population in the ovary. As each follicle supports and nurtures the development of one oöcyte, the population of follicles established during development represent the sole irreplaceable source of oöcytes for the reproductive lifespan of the female.

Growth of the Oöcyte

A primary oöcyte is distinguishable from other cells in the ovary by its large size, being about 35 µm in diameter in the human. An, as yet, unidentified signal triggers the initiation of growth of

the primordial follicle. The first signs of growth are an enlargement of the oöcyte while the surrounding somatic cells, now termed *granulosa cells*, assume a cuboidal shape and begin to proliferate. The human oöcyte reaches a final diameter of 120-140 µm which represents an approximate 1000-fold increase in cell volume.

Early after the initiation of oöcyte growth, patches of amorphous filamentous material appear between the oöcyte and the granulosa cells, eventually they completely surround the oöcyte forming the *zona pellucida*; its thickness increases with oöcyte growth. The zona pellucida is associated with all mammalian oöcytes, it forms a barrier, permeable to large macromolecules, between the oöcyte and the granulosa cells. Its main role appears to be at fertilization where it is responsible for the species-specific recognition of spermatozoa and also for triggering the acrosome reaction. The mouse oöcyte zona pellucida contains three glycoproteins ZP1, ZP2 and ZP3 all of which are produced by the oöcyte (Wassarman 1990 for review).

Although separated by the zona pellucida, the oöcyte and granulosa cells are dependent on the presence of each other for normal growth and differentiation. Communication between the two cell types is maintained by granulosa cell processes that pass through the zona pellucida and form gap-junctions with the plasma membrane of the oöcyte (Zamboni 1972; Anderson & Albertini 1976). Although not clearly established in the human, amino acids, other small metabolites, and regulatory molecules are known to pass between oöcytes and granulosa cells of other species (Moor et al, 1981; Heller, Cahill & Schultz 1981). This metabolic coupling allows the granulosa cells to contribute to the nutrition and regulation of the oöcyte. The oöcyte and granulosa cells are, however, interdependent and in the absence of an oöcyte the granulosa cells fail to develop further. This results in a 'streak ovary', seen in Turner's syndrome if the oöcytes degenerate, or in degenerating follicles if oöcytes are lost at later stages of folliculogenesis. Recent studies have demonstrated the importance of secreted factors from the oöcyte in regulating the proliferation and differentiation of the granulosa cells.

As the granulosa cells increase in number and form several layers, the oöcyte becomes increasingly isolated from the systemic and somatic influences of the ovary. During the growth of the follicle it becomes enveloped by a further layer of compressed, elongate cells thought to be derived from the ovarian stroma, the *theca folliculi* (3.20), which is separated from the granulosa cells by a basal lamina. At the time of antrum formation the theca differentiates into two layers, the *theca interna* and *theca externa*.

Cytological Changes During Oöcyte Growth

The nucleus of the primary oöcyte is relatively large, vesicular, usually eccentric, with a prominent nucleolus (3.20, 21). The distinctive nature of the nucleus is reflected in the more common name *germinal vesicle*. The *oölemma* (plasma membrane) of small oöcytes is smooth and in close apposition with the surrounding pregranulosa cells. As oöcyte growth continues and the zona pellucida increases in thickness, a uniform cover of microvilli develops. In non-growing primary oöcytes, organelles are relatively sparse and are concentrated in the juxtannuclear region. As oöcyte growth progresses, the number and size of organelles increase

and they disperse from the juxtannuclear zone through the cytoplasm. The Golgi apparatus is well developed and consists of parallel cisternae and numerous small vesicles; the latter also appear throughout the cytoplasm. Juxtannuclear annulate lamellae occur interspersed between spherical or slightly elongate mitochondria (Zamboni 1972^[1]). At first the endoplasmic reticulum is vesicular and displays few ribosomes which increase during oöcyte growth. Granular endoplasmic reticulum and free ribosomes, though not prominent, also increase. Lipid granules begin to appear; they are thought to correspond to the yolk platelets of earlier vertebrates but are smaller and less frequent in primates. Soon after oöcyte growth is initiated *cortical granules* begin to form from the Golgi apparatus; they are 500 nm electron dense granules, bound by a membrane (Gulyas 1980^[2]). As oöcyte growth nears completion the granules are distributed around the cortex in close proximity to the plasma membrane. The cortical granules are exocytosed at fertilization and are responsible for modifying the zona pellucida and plasma membrane to prevent further sperm penetration (Wassarman 1990^[3]).

Meiotic Divisions of the Oöcyte

From about 12 weeks gestation the oöcytes undergo a final series of DNA replication prior to entering the first meiotic division. Note that prophase of the first meiotic division is subdivided into five successive stages: leptotene, zygotene, pachytene, diplotene and diakinesis. During pachytene of first meiosis, the homologous chromosomes form bivalents and it is at this time that chiasmata form and recombination takes place (see p. 60^[4]). At entry into the final stage of the first meiotic prophase, *diplotene*, the pairing relaxes and the bivalents begin to separate, a process termed *desynapsis*. Oöcytes are arrested at diplotene, in the first meiotic prophase; they remain in this state, from about 20 weeks gestation, until stimulated to mature many years later. In rodents the chromatin becomes very diffuse, a state referred to as *dictyotene*. During dictyotene the bivalents separate further until they can no longer be traced, although some connections may remain. Thus the fully grown primary oöcyte contains the *diploid* number of double-stranded chromosomes and has been arrested at the diplotene (dictyotene in rodents) stage since before birth (Manotaya & Potter 1963^[5]; Ohno & Smith 1964^[6]).






The stimulation to resume meiosis is the midcycle surge of luteinizing hormone (LH; see p. 1866^[7]) which results in a number of intracellular changes first signified by the disappearance of the nucleolus and followed rapidly by the dissolution of the nuclear membrane. Homologous chromosomes have undergone condensation along the inner margin of the nuclear envelope and become arranged in homologous pairs at the equator of a spindle in the cortex of the oöcyte. The poles of the spindle in oöcytes lack centrioles and are composed of pericentriolar material (Szollosi 1972^[8]). As anaphase approaches, the homologous pairs of chromosomes move towards the poles of the spindle. A bulge forms at the site of the spindle which is destined to become the *first polar body*. The midbody forms around the spindle and initiates cleavage of the first polar body and final separation of the homologous chromosomes occurs. Unlike the equal division of the nucleus, the division of the cytoplasm is highly **unequal**, the polar body carrying with it its numerically equal chromosomal complement and an exiguous share of the cytoplasm. (The first polar body is occasionally seen to cleave into two equal fragments before degenerating sometime after ovulation.) The oöcyte resulting from this reduction division is known as the *secondary oöcyte*; it contains 23 double-stranded chromosomes (3.19^[9]). In the absence of an interphase

the chromosomes are rearranged around the equator of a second spindle. At metaphase of the second meiotic division the secondary oöcyte arrests until fertilization or parthenogenetic activation stimulates the completion of meiosis which is marked by extrusion of the *second polar body*.



The maturing primary oöcyte can produce some mRNA and rRNA. Its 'maternal' mRNA stores sustain it as it synthesizes various peptides during its final maturation from diakinesis through metaphase 1 to metaphase 2 in a period of about 37 hours. Meiotic maturation is regulated by a series of cell-cycle regulatory proteins similar to those in somatic cells. Resumption of meiosis and entry into metaphase I is associated with an increase in the activity of maturation promoting factor, a combination of *p34^{cdc2}* and cyclin B. At exit from metaphase I and entry into the second meiotic division, there is a transient decrease in maturation promoting factor activity. Arrest of the oöcyte in metaphase II is associated with the stabilization of *maturation promoting factor* by *cytostatic factor*, a component of which is the product of the *c-mos* proto-oncogene. Fertilization triggers the destruction of maturation promoting factor and cytostatic factor allowing the oöcyte to re-enter the cell-cycle, resulting in completion of meiosis and entry into G₁ of the first mitotic division.




Since the sperm delivers its haploid set of chromosomes prior to the completion of meiosis, the mammalian oöcyte is **not** technically an *ovum* as it is never a haploid cell. This is different to other species, e.g. sea-urchins, where fertilization occurs after the oöcyte has extruded the second polar body and the ovum contains a single haploid pronucleus. Nevertheless, the term remains widely used for many stages of mammalian oögenesis and embryogenesis.

Development of the Antral Follicle and Ovulation

Changes within the granulosa cell population, which form the primordial follicle, are concomitant with oöcyte growth. As the multilaminar follicle grows, a fluid-filled antrum appears among its proliferating cells, gradually dividing them into an internal stratum, the *cumulus oöphorus*, and an external *stratum granulosum*. At one site the two populations maintain continuity (3.20 ) and are connected via an extensive network of gap-junctions (Gilula et al 1978 ; Larsen et al 1986 ). The fully grown primary oöcyte is situated in the cumulus. As the antrum expands with *liquor folliculi* (follicular fluid) the granulosa cells form an envelope around the antrum which gradually thins to about 5–6 cells (Zamboni et al 1972 ). The granulosa cells closest to the basement membrane are columnar in shape while those closest to the antrum are polygonal. In the granulosa cell layer there is an increasing abundance of mitochondria, granular endoplasmic reticulum and free ribosomes and their Golgi organelles become more prominent, indicating increasing steroidogenic activity. The primary oöcyte, which remains in the prolonged prophase of the first meiotic division, retains its microvilli and its complex interrelationship with the cumulus cells outside the zona pellucida (3.21 ). The cells immediately surrounding the oöcyte within the cumulus oöphorus are known as the *corona radiata*.







Ovulation is initiated by a surge of gonadotrophins which also stimulates the resumption of

meiosis in the oöcyte. Just prior to ovulation, about 36 hours after the LH surge, the oöcyte extrudes the *second polar body* and arrests at metaphase of the second meiotic division. At this stage the oöcyte is termed the *secondary oöcyte* (3.22 ) and is surrounded by a clear *perivitelline* space beneath the zona pellucida. Leading up to ovulation the processes of the cumulus cells are withdrawn from the zona. Coincident with the breakdown of the cumulus cell processes there is a decrease in gap-junctions throughout the follicle (Larsen et al 1986 ) and the cumulus oöphorus expands in a hyaluronic acid matrix.

Soon after the LH surge, the preovulatory follicle becomes hyperaemic and oedematous. A small area of the follicle and overlying ovarian cortex becomes thin and translucent. As ovulation ensues the surface of the ovary bulges and eventually tears, liberating the secondary oöcyte surrounded by the zona pellucida and corona radiata. The mechanism responsible for the thinning of the follicle wall is not precisely known but is thought to involve the activation of collagenolysis through increased plasmin production as well as serine proteases; acting together the tensile strength of the follicle wall is reduced and rupture occurs (Lipner 1988 ). The released secondary oöcyte and expanded corona radiata is rapidly collected from the peritoneal cavity by the fimbria of the oviduct and carried into the infundibulum by ciliary movements of its epithelium (Austin 1963 ). Unless fertilization occurs, the secondary oöcyte is discharged from the uterus in the debris of the next menstrual period: if it is fertilized, the zygote which results is retained and pregnancy begins. (For details of fertilization see p. 132 )

Oöcyte Maturation in Vivo and in Vitro

Meiotic maturation refers to the development of the oöcyte from diplotene of meiosis I to metaphase II, where the oöcyte arrests, ready to undergo fertilization (see above). In addition to the nuclear events of maturation, the oöcyte becomes able to support the normal events of fertilization. This process is generally referred to as *cytoplasmic maturation* but very little is understood about the biochemical or molecular basis of these changes.

In vivo the preovulatory surge of gonadotrophins triggers meiotic maturation leading to germinal vesicle breakdown and progression to metaphase II (see above). Alternatively, mammalian oöcytes have the property that they can be stimulated to resume meiosis in vitro simply by removal of the oöcyte from the antral follicle (Pincus & Enzman 1935 ; Edwards 1965 ). This observation shows that the follicle is responsible for maintaining the oöcyte in meiotic arrest, although the mechanism remains unclear. Due to the ability of cAMP to inhibit meiotic maturation in vitro (Cho et al 1974 ) much attention has been paid to its role as the endogenous regulator of meiotic maturation (see Schultz 1986 ). The signal transduction mechanisms by which the surge of gonadotrophins relieves the inhibition of meiosis is also unclear. Several mechanisms are possible. The LH surge may serve to deprive the oöcyte of inhibitory signals from the follicle, for example by causing the inhibition of gap-junctional communication. Alternatively it may generate a positive signal in the follicular cells that overrides follicular inhibition. Recent work favours the latter hypothesis since hormones can override cAMP maintained meiotic inhibition in vitro (Dekel & Beers 1978 ; Downs et al 1988 ) and because gap-junctions between the oöcyte and follicular cells remain until after meiotic maturation has

resumed (Moor et al 1981^[1]; Eppig 1982^[2]).

The ability to undergo the normal events of fertilization is not a feature of all mammalian oocytes. This property is acquired during meiotic maturation. Immature oocytes with an intact germinal vesicle can be penetrated by sperm but fail to decondense the sperm head (Iwamatsu & Chang 1972^[3]). The ability to support full sperm head decondensation and pronuclear development is maximal once the oocyte is arrested at metaphase II. Thus the final stages of oocyte maturation are critical for the oocyte to undergo fertilization and development. These changes apparently occur normally in vitro as some oocytes matured in vitro have full development potential (Schroeder & Eppig 1984^[4]; Lu et al 1988^[5]; Cha et al 1991^[6]), although viability is generally lower than after maturation in vivo. The presence of follicle cells during meiotic maturation is necessary for normal cytoplasmic maturation (Stagmiller & Moor 1984^[7]) but the precise nature of oocyte modifications and how they may be influenced by the somatic environment are unclear.

Male Gamete

Gametogenesis in the male exhibits both marked similarities and differences in comparison with the development of ova. During maturation there is the same reduction of chromosomes to the haploid number and genetic recombination, but in the testis there is a continuous formation of spermatocytes and spermatozoa during reproductive life, linked with the enormous number of gametes which are formed. In each ejaculate there are many times more spermatozoa than there are germ cells in both ovaries at their peak content before birth; whereas the latter is of the order of 10 to 12 million, a single ejaculation may contain 300 million spermatozoa, only one of which may fertilize an ovum. (The nomenclature of male gametes is not yet unified officially; spermatozoön, spermatoid, sperm and spermium are all used.)

Morphology of Spermatozoa

A spermatozoön, or sperm (Rothschild 1957^[8]; Fawcett 1961a^[9], 1975^[10]; Pikó 1969^[11]), is a smaller cell than an oocyte, highly specialized to reach the latter and to carry to it its own haploid chromosome complement. Its expanded *caput* or *head* contains little cytoplasm and is connected by a short constricted *cervix* or *neck* to the *cauda* or *tail*. The latter is a flagellum of complex structure, usually divided into *middle*, *principal* and *end parts* or *pieces*. Volumetrically the tail much exceeds the head, which varies greatly in different species (Rothschild 1957^[8]; Phillips 1975^[12]), being ovoid or piriform in man, somewhat flattened at the tip in lateral profile, with a maximum length of about 4 µm and a maximum diameter of 3 µm. The tail, about 45–50 µm in length, displays a greater uniformity between species.

Head



(3.23 )

This is an extreme example of chromatin concentration, consisting largely of a dense and visually uniform nucleus, with a distinct bilaminar nuclear membrane and a bilaminar *acrosomal cap* (head cap), the latter covering the terminal two-thirds of the nucleus and partly derived from the spermatid Golgi apparatus. The acrosomal cap is thin in a human spermatozoön but in other species it is often large and more complex in shape. The acrosome has been shown to contain several enzymes including acid phosphatase, hyaluronidase and a protease (*acrosomase*), which are probably involved in penetration of the oöcyte. The nucleus and acrosome are enveloped in a continuous plasma membrane without intervening cytoplasm (Fawcett & Burgos 1956^[1]; Anberg 1957^[2]). The chromatin is stabilized by disulphide bonds, as if to protect its genetic content during the spermatozoön's journey (Fawcett 1975^[3]). So densely packed is the chromatin that it appears homogeneous even under electron microscopy. It has a strong affinity for basic stains, consisting of about 40% (dry weight) deoxyribonucleic acid and a protein rich in arginine (Daoust & Clermont 1955^[4]). It is also resistant to physical stress, e.g. ultrasonication (Henle et al 1938^[5]), and to mechanical shear (Mann 1949^[6]). Defects in condensation of nuclear material may be visible under light microscopy as relatively clear areas or *nuclear vacuoles*. Attempts to discover structural details in the nucleus in a variety of species, by polarization microscopy, X-ray diffraction and freeze-fracturing techniques, have shown a lamellar structure which cannot yet be equated with chromosomal content. The human Y chromosome has been identified using fluorescence microscopy (2.53^[7]).



Between the head and the middle part of body of the spermatozoön is a slight constriction, the *neck*, about 0.3 µm long. In its centre (3.23^[8]), close to a shallow recess in the base of the nucleus, is a well-formed centriole, corresponding to the *proximal* centriole of the spermatid from which the spermatozoön differentiated (3.25^[9]). The axial filament complex (axoneme) is derived from the *distal* centriole, a funnel-shaped *connecting piece* or *basal body* from which the outer fibrils of the tail extend (see below). (The *nuclear recess*, or *implantation fossa*, is the region of attachment of the complex filamentary structure of the tail. It is continuous with the postacrosomal part of the nuclear envelope concerned in fusion with the ovum and its nucleus.) A small amount of cytoplasm exists in the neck, covered by a plasma membrane continuous with that of the head and tail.

Middle Part or Piece


A long cylinder, about 1 µm in diameter and 7 µm long, it consists of an *axial bundle of microtubules (fibrils)* or *axoneme* (the axial 'filament' of light microscopy), surrounded by a *mitochondrial sheath* in which the mitochondria of the spermatid have become arranged in a helical manner (3.23B^[10]), the whole being enveloped by cytoplasm and a plasma membrane, as in the neck. The axoneme consists of a central pair of microtubules within a symmetrical set of nine doublet microtubules, as in a typical cilium (p. 41^[11]), and outside this is a second ring of nine coarser fibres, less symmetric in arrangement and unequal in size. These external fibres are also less regular in cross-sectional profile (see below), showing marked interspecific variations. They appear to be non-contractile despite showing a surface striation. Their function is obscure. The mitochondrial helix exhibits 10–14 turns (Reed & Reed 1948^[12]) but this sheath is subject to considerable variation in abnormal spermatozoa (Fujita et al 1970^[13]). The number of


mitochondria seems excessive in some species, including *Homo sapiens*, when related to the energy requirements of the axonema. Their close relation to the external coarse fibres is suggestive but, as noted, these are apparently not contractile. At the caudal end of the middle part of the cell, immediately anterior to the tail, is an electron-dense body, the *annulus* (3.25 ). The mitochondria of the sheath are much compressed, but it is now certain that they retain their individuality (Fawcett & Ito 1965 .

Principal Part or Tail

This is the motile part of the cell. Being about 40 µm long and 0.5 µm in diameter, it forms the greater part of the spermatozoön. The axial bundle of fibrils and the surrounding array of coarse fibres are continued uninterruptedly from the basal body through the mitochondrial sheath and through the whole length of the tail except for its terminal 5–7 µm, in which the axial bundle alone persists, the coarse fibres ceasing before them. It is only in this terminal *end part* or *piece* that the tail has the typical structure of a flagellum; the coarse fibres are peculiar to mammalian spermatozoa, which also display other specializations. External to the fibres and fibrils, coarse and fine, is a circumferentially orientated dense *fibrous sheath*, whose individual elements branch and reunite to form a tight reticulum. A small amount of cytoplasm and a plasma membrane complete the major elements in the structure of the tail. The finer details of the structure have been studied intensively in mammals such as the guinea-pig (Fawcett 1965  and, while there is little doubt that the human spermatozoön is highly similar (Pedersen 1969 , the descriptions presented here are based perforce on appearances in the guinea-pig.

Spermatogenesis

This is the complex series of changes by which spermatogonia are transformed into spermatozoa, similar in some general features—particularly in reduction division and genetic recombination—to the evolution of ova from oögonia, but differing in the more profound morphological metamorphosis involved. Spermatogenesis may, for convenience, be divided into three phases. During the first, *spermatocytosis*, spermatogonia proliferate by mitotic division to replace themselves and to produce primary spermatocytes. In the second phase, *meiosis*, two successive maturation divisions, the first a true reduction division as in the case of the oöcyte, produce *secondary spermatocytes* and then *spermatids*, all with the haploid number of chromosomes. In the third phase, *spermiogenesis* or *spermateliosis*, the spermatids become spermatozoa; it is during this period that the greatest visible transformation of structure occurs (3.24 .

Spermatogenesis is an orderly and complex sequence of events, with characteristic time constants and cell associations for each mammalian species. The details of these in the human testis will be discussed with that organ (p. 1851 .

Spermatocytosis

During embryonic, fetal and perhaps also early postnatal life, *primordial germ cells* in the

tubules of the testes divide mitotically to produce spermatogonia (Witschi 1948^[1], 1951^[2]; Mintz 1960^[3]), from which, at and subsequent to puberty, the development of spermatocytes and spermatozoa commences. It is the cyclic divisions of these cells, the details of which have attracted much attention in recent years, that form the starting place for production of the huge numbers of spermatozoa discharged into the seminal plasma to form the seminal fluid. The series of changes involved do not occur in a synchronous manner in all seminiferous tubules at the same time, although they do in considerable parts of an individual tube, with variations in different mammalian species, including mankind. As the cycle of change from spermatogonia to spermatids proceeds at any particular locus in a tubule, a succession of varying cell associations can be observed and measured; the process is termed the *cycle of the seminiferous epithelium* (Clermont & Leblond 1955^[4]) (p. 1852^[5]).

In man, three types of spermatogonia can be distinguished and are termed: the *dark type A*, *light type A* and *type B* (Clermont 1963^[6]). Spermatogonia are large rounded cells, the three types showing little difference in size or in their cytoplasm, but the A series, light and dark, are distinguishable by their nucleoli which are eccentric and attached to the internal aspect of the nuclear membrane. The type B spermatogonia have a more constantly spherical nucleus, in which the nucleolus is central in position. The dark type A is distinguished from the pale type A by its dark nucleoplasm and a large pale-staining nuclear vacuole. The dark type A is now considered, largely on morphological grounds, to be the progenitor or stem spermatogonium (Clermont 1963^[6]). Such cells, peripherally situated in the tubule and often in pairs, divide mitotically at the beginning of a seminiferous cycle, some to produce two further dark type A spermatogonia, thus replenishing the complement of stem cells, others into two light type A spermatogonia. Mitotic division of a light type A spermatogonium furnishes two type B spermatogonia. On theoretical grounds, it is probable that in man a larger series of spermatogonial divisions may occur, so that the ultimate spermatocyte progeny of a stem cell may in fact be more numerous than is here indicated (Clermont 1966^[7]). Each type B spermatogonium then divides again mitotically into two *resting primary spermatocytes* or *preleptotene spermatocytes*. The dark type A cells remain arranged along the basal lamina of the tubule, whereas the pale type A, type B spermatocytes and the spermatids derived from them lie closer to the lumen, into which the free end-product, spermatozoa, will be discharged. These events constitute spermatocytosis, which is now followed by meiosis.

Meiosis of Spermatocytes

The primary spermatocytes soon enter the prophase of the *first maturation (reduction) division*, which is prolonged over several days through the successive stages of leptotene, zygotene, pachytene, diplotene and diakinesis (p. 61^[8]) (Clermont 1963^[6], 1966^[7]). As the nuclear membrane now disappears in metaphase, the bivalent chromosomes are arranged on the equatorial plate, separating into two groups and moving to opposite poles in anaphase, followed in the usual manner by reformation of the nuclear membranes in telophase and division of the cell. These three phases occur much more rapidly than prophase and during the whole process there is a considerable increase of nuclear and cytoplasmic material, bringing the primary spermatocyte back to a size comparable with that of the stem spermatogonium. The two *secondary spermatocytes* thus formed contain, of course, the haploid number of chromosomes,


this *first* maturation division being the one which is strictly speaking *meiotic*. After a brief interphase each secondary spermatocyte now undergoes a *second maturation division*, which is by mitosis. The two resultant cells are spermatids and with their formation the phase of meiosis may be considered to end, being followed by their maturation into spermatozoa (spermiogenesis). Theoretically each primary spermatocyte may be expected to produce four spermatids but in mankind the yield is less than this, presumably because some spermatocytes degenerate during maturation.



Criticism of the traditional view that spermatids do not divide has been expressed (Roosen-Runge 1952^[1]) and tentative corroboration of this came from electron microscope and other studies (Fawcett et al 1959^[2]; Fawcett 1961b^[3]). These interpretations have subsequently been subjected to critical scrutiny. Electron microscopy has also shown that the division of the cell body (cytokinesis) in spermatocytes may be delayed, so that fine cytoplasmic bridges remain, interconnecting such cells even beyond the stage of the next nuclear division (Fawcett et al 1959^[2]). These bridges, which may remain in the case of spermatids until a late phase in their transformation into spermatozoa, are short, devoid of spindle fibres or other remnants and are enclosed in annular thickenings of the plasma membrane. They probably permit interchange of organelles, may be involved in synchronization of development and may contribute to the mechanical stability of the spermatid-Sertoli cell complexes. Such cytoplasmic interconnection may also help to explain the formation of multinucleated masses when the seminiferous epithelium is injured, as in making teased preparations. Except where connected together by bridges the developing spermatids are very closely associated with Sertoli cells, whose processes are insinuated between them.


During the differentiation of some rodent spermatids a fusiform conglomeration of microtubules, the 'spindle-shaped body', appears between the annulus and fibrous sheath. The same structure has been observed in human spermatids; its functional significance is uncertain but an association with the development of the fibrous sheath has been suggested (Pedersen 1969^[4]; Wartenberg & Holstein 1975^[5]).


Spermiogenesis


During *spermiogenesis* (spermateliosis), spermatids go through a complex series of changes to become spermatozoa (3.25^[6]) and this metamorphosis has been studied in particular by light microscopy, using preparations stained by the periodic-acid Schiff technique (PAS); (Clermont 1963^[7]). Electron microscopy has confirmed these observations (Fawcett & Burgos 1956^[8]). In the newly formed spermatid the Golgi apparatus (idiosome–Golgi complex) is large but otherwise typical, consisting of flattened membrane-enclosed vesicles, usually stacked in a parallel array, together with rounded minute vesicles which are possibly nipped off from the flattened variety, the whole complex being juxtannuclear. A few electron-dense homogeneous *paracrosomal granules*, which are intensely PAS-positive, develop in separate Golgi complex vesicles and the latter coalesce into a single large *acrosomal vesicle*, the separate granules fusing into a single spherical *acrosomal granule*. This vesicle, with its granule attached to its juxtannuclear wall, becomes adherent to the nuclear membrane over an area which will be anterior or 'leading' in the maturing spermatozoön. The granule flattens, but its central part

bulges slightly into a shallow depression in the nucleus, which becomes progressively more ovoid (3.25 ). By successive absorption of further vesicles from the Golgi complex, the material in the acrosomal vesicle increases and the vesicle expands as a bilaminar cap over the anterior two-thirds of the nucleus. Coincident with these changes, the spermatid elongates and the Golgi complex and associated cytoplasm migrate to the posterior part of the cell, bringing the external wall of the acrosomal vesicle into contact with the plasma membrane at the anterior aspect of the cell. The acrosomal granule now spreads out between the layers of the vesicle until it is uniformly distributed and no longer a localized structure. When the centrioles begin to separate (see below), microtubules develop forming an inverted conical array, the *manchette*, perinuclear in position and expanding from the region of the acrosomal cap; its precise significance is not yet explained.

In the early spermatid the nucleus is of relatively low density, containing finely dispersed granules which aggregate into larger and denser masses as development proceeds. These finally agglomerate into a homogeneously dense mass, usually containing one or more regions of low electron-density and variable in size, position and shape—the *head vacuoles* (Fawcett & Burgos 1956 ). Correlated biochemical and ultrastructural studies indicate a considerable variation in the chromatin content of spermatozoa, this heterogeneity being more marked in mankind than other primates or rodents; it probably indicates a lower fertilizing power (Bedford et al 1973 .

The two centrioles are near the posterior aspect of the nucleus from an early stage. One remains unmodified; the other becomes modified into the basal body of the spermatozoön (see p. 125 ). The annulus arises close to the latter (the distant centriole) but its origin from it is doubtful. The axial fibrils begin to develop from the basal body, extending 'caudally' into the cytoplasm of the cell as it becomes progressively more elongated. Only the proximal part of the bundle of fibrils remains surrounded by cytoplasm, to form the definitive middle part of the spermatozoön. In this, the mitochondria of the spermatid assemble to form the helical sheath. The detailed development of the fibrous sheath of the tail part is unknown. These changes complete what might be called the period of 'organogenesis' of the spermatid, whose further development into a spermatozoön is largely concerned with enlargement of the tail.

During the final maturation of spermatids into individual spermatozoa some of the cytoplasm is detached as a *residual body*. This contains some mitochondria, Golgi membranes and vesicles, RNA particles, lipid granules but of course no nucleus. Residual bodies are prominent when spermatozoa are being released into their tubule. They are engulfed by Sertoli cells, which accounts for the increase in lipid content of these cells at this period (Lacy 1960 .

As already stated, there is a close relation between developing spermatids and Sertoli sustentacular cells. The spermatogonia are external or basal to the Sertoli cells in the tubule and the spermatocytes which develop from the former are embraced by Sertoli processes; the spermatids are even more deeply embedded in the supportive cells. (For further details see p. 1852 .) These associations have been regarded as symbiotic, a single sustentacular cell being grouped with several spermatids. The Sertoli cells are phagocytic and absorb not only residual bodies but also degenerating germ cells. They have been attributed a metabolic role and may form, or at least transmit, hormones involved in the maturation of germ cells. Until released into

the seminiferous tubule, spermatozoa are very firmly held by the Sertoli cells. Their release is sometimes termed *spermiation* and is followed by rapid translation of the spermatozoa to the epididymis.

Maturation of Spermatozoa

Maturation is a complex process which has received much attention. Spermatozoa show little independent motility while still in the male genital tract, though when removed from the epididymis they may display circular swimming movements or even directive movements if taken from the cauda epididymis near the beginning of the ductus deferens (Blandau & Rumery 1964^[1]). From the results of artificial insemination of rabbits with spermatozoa from the caput and cauda of the epididymis, it has been postulated that some form of maturation process takes place in this organ, during which the spermatozoön attains its specific pattern of motility (Gaddum 1968^[2]). Apart from these incomplete activities, spermatozoa are largely transported through the genital tract by ciliary action, by fluid currents set up by localized secretion and absorption and by muscular contractions. Associated with the maturation of spermatozoa in the genital tract in some mammals is the extrusion of a small mass of cytoplasm, the *kinoplasmic droplet* or *residual body*, which migrates backwards along the surface of the head and middle part of the spermatozoön before disappearing. It consists of membrane-enclosed cytoplasm containing fine tubules and vesicles (Bloom & Nicander 1961^[3]; Guraya 1963^[4]). Human spermatozoa have not been shown to undergo any demonstrable structural changes during passage through the epididymis, but there is evidence of an increase in sulphide cross-linking between proteins (Bedford & Calvin 1974^[5]). Moreover, restorative surgery after vasectomy indicates that at least part of the human epididymis is essential for motility (Bedford et al 1973^[6]).

Motility of Spermatozoa

In cross-section the tail is oval and tapers caudally and its central area is typical of a flagellum or cilium. The surrounding coarse fibres are obovate or petal-shaped and unequal in size, one being consistently the largest. This is given the number 1 and the rest are numbered from this in a clockwise manner. These fibres are separated into two unequal groups by slender *longitudinal* columns in the fibrous sheath which interrupts its circumferential fibres and extend inwards to meet the coarse fibres numbered 3 and 8. This divides the interior of the tail into *major* and *minor compartments*, containing respectively coarse fibres 4, 5, 6 and 7, and 9, 1 and 2. The plane through the two columns also passes through the central pair of the axial bundle of fibrils and can be used as a reference datum for other structural details. For example, the transverse diameter of the head has been considered to lie at right angles to the plane of the columns, but it has now been shown in the guinea-pig that the angle between the two planes is 20–30° less than a right angle (Fawcett 1968^[7]). Such details may be instrumental in elucidating the motile activities of the tail. It is now generally accepted that the tail executes undulatory movements in one plane, but it has also been suggested that a helical component is superimposed upon this, there being perhaps two separable mechanisms, one involving flat waves travelling along the tail, the other associated with torsional activity (Gray 1958^[8]; Bishop 1962^[9]; Lindahl & Drevius

1964^[4]).

The latter variety of movement has been linked with the unequal size and distribution of the coarse fibrils; it has also been suggested that the central pair of fine fibrils act as axial stiffeners. The asymmetry of the spermatozoan head has also been invoked to explain supposed helical movement. However, it has to be admitted that the full details of the mechanisms of spermatozoal motility are unknown.

Some further details deserve mention. The dense outer fibres have been shown to exhibit an oblique or helical striation in replicas of dried whole mounts of rodent spermatozoa (Phillips & Olson 1975^[4]). The central axoneme (3.23^[4]), consisting of the usual ciliary pattern of nine double fibrils or 'doublets', has been intensively studied (Fawcett 1975^[4] for survey of literature). Each fibril is a microtubule, itself constructed of a regular number of protofibrils. Protein bridges connect adjoining doublets at regular intervals and radial links extend centrally to the central doublet of the axoneme.

As soon as they are ejaculated the spermatozoa display their full pattern of motility. The precise factors which trigger off these movements enabling human gametes to travel at a rate of 1.5–3 mm/min are not yet clear; but the other constituents of semen, derived from the epididymis and testis and from the seminal vesicle and prostate, are generally considered to exert an activating influence. The motility varies greatly in different species, disappearing in minutes in some fish but usually persisting in mammals for hours and even days when introduced into the female genital tract. Exact figures for its persistence in the human female are uncertain and are of doubtful value, since it is likely that, as in other mammals, human spermatozoa quickly lose their potency for fertilization, although still motile. They have been recovered in a motile state in human cervical mucus several days after insemination and will survive in this condition for as long as 7 days when implanted into such secretions *in vitro*. These survival periods may, however, be of little significance, in view of the speed with which spermatozoa reach the infundibulum of the uterine tube and the brevity of their fertilizing power. Spermatozoa have been shown to reach their tubal destination in a manner of minutes after ejaculation in some mammals and experiments on recently excised human uteri and tubes indicated a time of about 70 minutes (Brown 1944^[4]). The conclusion must be that factors other than their own motility are responsible for the transport of spermatozoa from the site of deposition in the vaginal fornix to the ovarian end of the uterine tube and there is considerable evidence that contraction of the uterine and tubal musculature is responsible (Bickers 1960^[4]).

It is not usually recognized that a spermatozoön must be adaptable to a wide range of environments in its long journey from the seminiferous tubule to the uterine tube, encountering major changes in the electrolyte and non-electrolyte constituents in the fluids with which it is successively surrounded. Nevertheless, a collectively vast amount of observation and experiment has been recorded in connection with the effects of the multitude of factors, both physical and chemical, in the natural media involved regarding the behaviour of these cells and particularly their motility and fertility (Nelson 1967^[4]; Mann 1967^[4]); e.g. the effects of respiratory gas tensions, reaction, various ions, antibodies, vitamins, hormones, inhibitory substances, temperature, different forms of radiation and other factors have been studied in remarkable

detail, for which monographs and original papers must be consulted. The effects of low temperatures in preserving spermatozoa and perhaps prolonging their vitality have attracted much research in connection with artificial insemination, both in stock-breeding and in infertile human marriage. Mammalian semen, including that of human beings (Parkes 1952^[1]), can be stored at temperatures of about -70°C for weeks and even months, the motility and fertility of its suspended spermatozoa reappearing when the suspension is unfrozen. However, storage of human semen presents difficulties (Polge 1957^[2]).

Seminal Plasma

The fluid component of *seminal fluid* or *semen*; it contains a remarkable array of substances, including muco-proteins, a dozen or more identified proteolytic enzymes, the bases spermine, glycerylphosphorylcholine and ergothioneine, a group of organic acids called prostaglandins (which have pharmaco-dynamic actions on the uterus and smooth muscle in general), acids such as citric, ascorbic, uric, lactic and pyruvic, and the sugars sorbitol, inositol and fructose. The fructose added to the fluid by the secretion of the seminal vesicle is an essential substrate in the anaerobic glycolysis by which spermatozoa survive the low oxygen tensions existing in semen itself and in the female genital tract. Prostaglandins are now believed to play a role, perhaps by modulation of neurotransmitter release, in the contraction/relaxation activity of the non-striated muscle in the testicular capsule and interlobular septa adjacent to the seminiferous tubules (as suggested by von Euler 1936^[3]). Consult Ellis and Hargrove (1977^[4]) for literature.

Capacitation

After ejaculation into the female, the spermatozoa undergo the final step in their maturation, a process known as *capacitation*. It has been shown that spermatozoa are not able to fertilize ova until they have been within the genital tract of the female for a period of time, usually of hours but varying with the species (Austin 1951^[5]; Chang 1951^[6]; Austin & Walton 1960^[7]). The mechanism of capacitation, whereby the spermatozoön is activated to enter and fertilize the ovum, is still uncertain. A confusing array of findings with regard to the interactions of the two gametes immediately prior to this event have been described, unfortunately in widely different vertebrates and invertebrates. It is probable that hyaluronidase hastens the separation of corona radiata cells from the ovum, and thus facilitates the spermatozoön's approach to the zona pellucida. The origin of hyaluronidase from the acrosomal cap is associated with subsequent loss of the cap (Leuchtenberger & Schrader 1950^[8]), at least in part (Austin & Bishop 1958^[9]). 'Capacitated' spermatozoa observed in the zona pellucida or perivitelline space have invariably lost most of their acrosomal material (Leuchtenberger & Schrader 1950^[8]; Pikó & Tyler 1964^[10]) and it is clear that capacitation is some process of activation which precedes penetration. Antigenic 'coating' substances on the surface of mammalian spermatozoa, including those of man (Weil 1965^[11]), have been recorded and it is possible that an immunological reaction may be involved. Interaction between a *fertilizin*, derived from the ovum or elsewhere in the female genital tract, and a spermatozoan *anti-fertilizin* has been associated with capacitation, but the interrelationship between the various events is still sub judice, as comprehensive reviews show (Metz & Monroy 1969^[12]; Chang & Hunter 1975^[13]). Capacitation may be regarded as the terminal

event of maturation of the spermatozoön, prior to actual fertilization, for which it is preparation.

Union of the Gametes

Sexual reproduction is initiated by the fusion of distinct male and female gametes (sperm and egg respectively) produced by appropriately different parental forms. In some of the earliest organisms to propagate by sexual reproduction, such as primitive algae, the gametes are all alike, except presumably in their genetic content. The profound differences which have evolved in the gametes of the great majority of plants and animals—vertebrate and invertebrate—appear to depend on a conflict between adaptation for the carriage of nutrients for one gamete and improvement in motility for the other. The egg, by extruding genetic material at the meiotic divisions, accumulates cytoplasm and is considered immobile; during its development the sperm loses cytoplasm retaining only the nucleus, mitochondria, a propulsive tail and such organelles necessary for the production of enzymes for breaching the outer walls of the egg. This dimorphism of the gametes has in turn entailed the evolution of equally profound differences between the individuals producing the two kinds of gametes, males and females, in regard to the organs concerned in bringing together these dissimilar gametes and ensuring the development of their fused product, the *zygote*, until it is able to undertake a separate existence.

The central feature of reproduction in most plants and animals is the *fusion* of the gametes at fertilization. Fusion is the precursor of *syngamy*, when the two gamete pronuclei come together to reconstitute a diploid *zygote* nucleus. Syngamy is the second and final stage of the genetic assortment that accompanies (and is, no doubt, the reason for) sexual reproduction; the first stage is meiotic crossing over (recombination). Genetic sex is determined at syngamy by the presence or absence of the Y chromosome that determines the male sex in mammals and several other animal groups. The Y chromosome, if present, is necessarily contributed by the sperm which therefore determines the sex of the zygote. In most animals, phenotypic sex is female, unless active genes on the Y chromosome are present to trigger the active programme for male sex determination.

Although syngamy is essential for the maintenance of ploidy, other changes within the egg that are equally essential for normal development are triggered by fertilization. Mammalian gametes are fertilized when in the second meiotic metaphase: fertilization causes the cell division cycle to resume, completing meiosis and extruding the second set of redundant meiotic chromosomes as the second *polar body*. Thereafter, cell division (segmentation or cleavage) proceeds within the zona pellucida until the blastocyst stage (Howlett & Bolton 1985📖).

Parthenogenesis

A mature egg can contain all that is necessary to make a new being and can under some circumstances commence to develop. This is particularly evident in the cases of *natural* and *artificial parthenogenesis*. Natural parthenogenesis occurs quite widely, a good example being aphids, which produce parthenogenetic females to maximize generation overlap and thus

population growth rate at times of food abundance, reverting to sexual reproduction when food is scarce. Artificial parthenogenesis stimulates this potentiality of the egg to develop further without fertilization by a variety of mechanical, chemical and physico-chemical means, such as pricking of the egg or exposure to altered tonicity and chemicals. It has been successful in a wide variety of animals, though not in mammals where offspring rarely survive beyond the embryonic stage (underlining the point that the genetic contribution of the gamete nuclei to early development is small, but becomes important later). In the case of rabbits, viable parthenogenotes have been reported, but the observation has never been substantiated.

Parental Imprinting

The presence of chromosomes from both parental origins is crucial for spatial organization and the controlled growth of cells, tissues and organs (Azim & Surani 1986^[1]). There is extensive evidence which suggests differential roles for paternal and maternal genomes during mammalian embryogenesis. Embryos in which the paternal pronucleus has been removed and replaced with a second maternal pronucleus develop to a relatively advanced stage, in the mouse, to form 25-somite embryos with very limited development of the trophoblast and extraembryonic tissues. In contrast, embryos in which the maternal pronucleus has been replaced by a second paternal pronucleus develop very poorly, forming embryos of only 6–8 somites, but with extensive trophoblast. Thus it seems that the maternal genome is relatively more important for the development of the embryo, while the paternal genome is essential for the development of the extraembryonic tissues.

This *functional inequivalence* of homologous parental chromosomes is called *parental imprinting*. The process of parental imprinting causes the expression of particular genes to be dependent on their parental origin, with some genes being expressed only from the maternally inherited chromosome and others from the paternally inherited chromosome. These genes are called *imprinted genes*, they are believed to have inherited maternal or paternal specific *imprints* which affect their activity (3.26^[2]) (Surani et al 1986^[3]; Solter 1988^[4]). The mechanism of imprinting is thought to involve the acquisition of molecular signals attached to the DNA or chromatin which are known as *epigenetic modifications*. These modifications must have the following properties:

- (1) They must be able to affect the transcription of the gene.
- (2) They must be heritable in somatic cells over many cell divisions and not lost during chromosome replication.
- (3) Most importantly, the imprints must be erased in the male and female germ lines during gametogenesis to allow new imprints to be set down which are specific to the parental origin of the newly formed gametes.

Methylation of some critical CpG dinucleotides in the DNA of imprinted genes is one type of epigenetic modification believed to be important in the imprinting process (Sasaki et al 1993^[5]).

The requirement for both parental genomes is limited to a subset of the chromosomes (Cattanach & Beechey 1994^[1]). This has become evident through the analysis of the individuals with uniparental duplications and corresponding deficiencies, *uniparental disomy*, of particular chromosomal regions. Uniparental disomy (3.27^[2]) can arise through meiotic and mitotic non-disjunction events and result in individuals completely disomic or exhibiting *mosaicism* of disomic and non-disomic cells. If imprinted genes reside on the affected chromosomes then the uniparental disomic cells will either express a double dose of the gene or have both copies repressed. For example, the gene encoding the embryonal mitogen insulin-like growth factor II is expressed from the paternally inherited chromosome and repressed when maternally inherited (DeChiara et al 1991^[3]). Thus individuals with maternal duplication/paternal deficiency, *maternal disomy*, of the chromosome carrying the insulin-like growth factor II gene do not express any of the growth factor. Mice with this deficiency are growth retarded (Ferguson-Smith et al 1991^[4]).


In humans, some conditions show parental origin effects in their patterns of inheritance and several imprinted disorders in man have been described. These disorders can be attributed to alterations in the dosage of imprinted genes either through chromosomal uniparental disomy, trisomy or mutations (e.g. deletions) involving the gene or the imprints. In these disorders, males and females are equally affected; however, manifestation of the disorder depends on the parental origin of the uniparental disomy or the sex of the parent from whom the mutation is inherited. Disorders exhibiting parental origin effects in their patterns of inheritance include the Beckwith-Wiedemann syndrome (Wiedemann 1964^[5]; Elliott & Maher 1994^[6]), Prader-Willi syndrome (Holm et al 1993^[7]) and Angelman syndrome (Angelman 1965^[8]). Parental imprinting mutations are also implicated in the genesis of some tumour syndromes, notably Wilm's tumour and familial glomus tumours.

Fertilization



Fertilization normally occurs in the ampullary region of the uterine tube probably within 24 hours of ovulation. Very few spermatozoa reach the ampulla to achieve fertilization. They must undergo capacitation, still incompletely understood, which may involve modifications in membrane sterols or surface proteins. They traverse the cumulus oöphorus and corona radiata, then bind to specific glycoprotein receptors on the zona pellucida, ZP3 and ZP2. Interaction of ZP3 with the sperm head induces the *acrosome reaction*, in which fusion of membranes on the sperm head releases enzymes, such as acrosin, which help to digest the zona around the sperm head allowing the sperm to reach the perivitelline space. In the perivitelline space, the spermatoöon fuses with the oöcyte microvilli, possibly via two disintegrin peptides in the sperm head and integrin in the oölemma. Fusion of the sperm with the oölemma causes a weak membrane depolarization and leads to a *calcium wave* which is triggered by the sperm at the site of fusion and crosses the egg within 5–20 seconds (see 3.28^[9]). The calcium wave amplifies the local signal at the site of sperm–oöcyte interaction and distributes it throughout the oöcyte cytoplasm. The increase in calcium concentration is the signal that causes the oöcyte to resume cell division initiating the completion of meiosis II and setting off the developmental programme that leads to embryogenesis. All vertebrate, and some invertebrate, eggs initiate a calcium wave

at fertilization.

The pulses of intracellular calcium that occur every few minutes for the first few hours of development also trigger the fusion of *cortical granules* with the oölemma. The cortical secretory granules release an enzyme that hydrolyses the ZP3 receptor on the zona pellucida and so prevents other sperm from binding and undergoing the acrosome reaction, thus establishing the *block to polyspermy*. The same cortical granule secretion may also modify the vitelline layer and oölemma, making them less susceptible to sperm–oöcyte fusion and providing a further level of polyspermy block.



The sperm head undergoes its protamine B histone transition as the second polar body is extruded. The two *pronuclei* grow, move together and condense in preparation for syngamy and cleavage after 24 hours (3.32 ). Nucleolar rRNA and perhaps some mRNA is synthesized in pronuclei, and a succeeding series of cleavage divisions produces eight even-sized blastomeres at 2.5 days, when embryonic mRNA is transcribed.

Several examples of cells, *oötids*, which contain male and female pronuclei have been described. Pronuclear fusion as such does not occur; the two pronuclear envelopes disappear and the two chromosome groups move together to assume positions on the first cleavage spindle. Thus there is no true zygote stage containing a membrane-bound nucleus.

Fertilization of human gametes in vitro (IVF) is very successful (3.28 ). Controlled ovarian stimulation, (e.g. with pituitary down-regulation with luteinizing hormone releasing hormone (LHRH); analogues followed by stimulation with menopausal gonadotrophins, enables many preovulatory oöcytes (often 10 or more) to be aspirated by laparoscopy or transvaginal ultrasound. Micromanipulation assists severe male infertility, especially by injecting a spermatozoön directly into the oöplasm to obtain 50% fertilizations. Genetic disease in embryos is being diagnosed by applying the polymerase chain reaction to polar bodies removed from oöcytes, or to one or more blastomeres excised from cleaving embryos or pieces of trophectoderm excised from blastocysts. Pronucleate and cleaving embryos are cryopreserved using propanediol or dimethylsulphoxide, and blastocysts using glycerol. Conception rates per cycle using ovarian stimulation, IVF and successive transfers of fresh and cryopreserved embryos far outstrip those obtained during non-assisted conception (Edwards & Brody 1993 .

Preimplantation Development

Cleavage

The first divisions of the fertilized oöcyte are termed cleavage. They distribute the cytoplasm approximately equally among daughter *blastomeres*, so although the cell number of the preimplantation embryo rises its total mass actually falls slightly (3.29 , 30 ). The cell cycle is quite long, the first two cell cycles being around 24 hours each, thereafter reducing to 12 to 18 hours. Cell division is asynchronous and daughter cells may retain a cytoplasmic link

through much of the immediately subsequent cell cycle via a midbody, due to the delayed completion of cytokinesis. No centrioles are present until the 16- to 32-cell stage, but amorphous pericentriolar material is present and serves to organize the mitotic spindles, which are characteristically more barrel than spindle shaped at these stages.

All cleavage divisions after fertilization are dependent upon continuing protein synthesis. In contrast, passage through the earliest cycles is independent of mRNA synthesis (to 2 cells in mouse, 4 cells in pig, 8 cells in human, 16 cells in cow and sheep), but thereafter the inhibition of transcription experimentally blocks further division and development, indicating that activation of the embryonic genome is required. There is also direct evidence for the synthesis of embryonically encoded proteins at this stage. At the same time as the embryo's genes first become both active and essential, the previously functional maternally derived mRNA is destroyed. However, protein made on these maternal templates does persist at least to the blastocyst stage. Interestingly, spontaneous developmental arrest of embryo culture in vitro seems to occur during the cell cycle of gene activation in all species studied including the human, but it is not caused by total failure of that activation process (Schultz 1993^[4]). The early cleavage stages, up to around the 8-cell stage, require pyruvate or lactate as metabolic substrates, but thereafter more glucose is metabolized and may be required (Leese 1991^[5]).


The earliest stage at which different types of cells can be identified within the cleaving embryo probably depends upon the species but tends to be around the 8- to 16-cell stage. It has been studied in most detail in the mouse embryo. Up to the early 8-cell stage of mouse embryogenesis, cells are essentially spherical, loosely touching each other, having no specialized intercellular junctions or significant extracellular matrix, and the cytoplasm of each being organized in a radially symmetric manner around a centrally located nucleus. During the 8-cell stage, the process of *compaction* occurs in which cells:

- flatten on each other to maximize intercellular contact
- initiate formation of gap and focal tight junctions
- radically reorganize their cytoplasmic organization from radially symmetric to a highly asymmetric phenotype.


This latter process includes the migration of nuclei towards the centre of the embryo, redistribution of surface microvilli and an underlying mesh of microfilaments and microtubules to the exposed surface and the localization of endosomes beneath the apical cytoskeletal mesh. As a result of the process of compaction, the embryo forms a primitive proto-epithelial cyst, with 8 polarized cells, their apices facing outward and their basolateral surfaces internally. The focal tight junctions, which align to become increasingly linear, are localized to the boundary between the apical and basolateral surfaces; gap junctions form between apposed basolateral surfaces and become functional (Fleming & Johnson 1988^[6]).

The process of compaction involves the cell surface and calcium dependent cell:cell adhesion glycoprotein E-cadherin (also called L-CAM or uvomorulin). Neutralization of its function disturbs all three elements of compaction. The whole process can function in the absence of both

mRNA and protein synthesis. Post-translational controls are sufficient and seem to involve regulation through protein phosphorylation. Significantly, although E-cadherin is not synthesized and present on the surface of cleaving blastomeres, it first becomes phosphorylated early during the 8-cell stage at the initiation of compaction.

The process of compaction is important for the generation of cell diversity in the early embryo. As each polarized cell divides, it retains significant elements of its polar organization so that its daughter cells inherit cytocortical domains, the nature of which reflect their origin and organization in the parent 8-cell. Thus, if the axis of division is aligned approximately at right angles to the axis of cell polarity, the more superficially placed daughter cell inherits all the apical cytocortex and some of the basolateral cytocortex and is polar, whilst the more centrally placed cell inherits only basolateral cytocortex and is apolar. In contrast, if the axis of division is aligned approximately along the axis of the cell polarity, two polar daughter cells are formed. Thus, *2-cell populations* are formed in the 16-cell embryo that differ in phenotype (polar, apolar) and position (superficial, deep), and the number of cells in each population in any one embryo will be determined by the ratio of divisions along and at right angles to the axis of 8-cell polarity. The theoretical and observed limits of the polar to apolar ratio are 16:0 and 8:8. The outer, polar cells contribute largely to the trophoctoderm whilst the inner, apolar cells contribute almost exclusively to the inner cell mass in most embryos (Johnson et al 1986.

In cleavage therefore, the generation of cell diversity, to either *trophoctoderm* or *inner cell mass*, occurs in the 16-cell morula and precedes the formation of the blastocyst. During the 16-cell cycle, the outer polar cells continue to differentiate an epithelial phenotype, displaying further aspects of polarity and intercellular adhesion typical of epithelial cells, while the inner apolar cells remain symmetrically organized. During the next cell division (16- to 32-cell stage), a proportion of polar cells again divide differentiatively as in the previous cycle, each yielding one polar and one apolar progeny that enter respectively the trophoctoderm and inner cell mass lineages. However, in this case, differentiative division is less common than at the 8- to 16-cell transition, yet has the important function of regulating an appropriate number of cells in the two tissues of the blastocyst. Thus, if differentiative divisions were relatively infrequent at the 8- to 16-cell transition, they will be more frequent at the 16- to 32-cell transition, and vice versa.

Following division to the 32-cell stage, the outer polar cells complete their differentiation into a functional epithelium, and display structurally complete zonular tight junctions and begin to form desmosomes. The nascent trophoctoderm engages in vectorial fluid transport in the apical to basal direction to generate a cavity which expands in size during the 32- to 64-cell cycles converting the ball of cells to a sphere, the blastocyst. By the blastocyst stage, the diversification of the trophoctoderm and inner cell mass lineages is complete and trophoctoderm differentiative divisions no longer occur. In the late blastocyst, the trophoctoderm is referred to as the *trophoblast*; it can be divided into *polar trophoblast* which lies in direct contact with the inner cell mass, and *mural trophoblast* which surrounds the blastocyst cavity (3.35.

Staging of Embryos

Prenatal life can be divided into an embryonic period and a fetal period. The embryonic period covers the first 8 weeks of development (weeks following ovulation and fertilization resulting in pregnancy). The ages of early human embryos have been previously estimated by comparing their development with that of monkey embryos of known postovulatory ages. Because embryos develop at different rates and attain different final weights and sizes, a classification of human embryos into 23 stages occurring during the first 8 postovulatory weeks was developed, most successfully, by Streeter (Streeter 1942^[1]); a task continued today by O'Rahilly (O'Rahilly & Muller 1987^[2]). An embryo was initially staged by comparing its development to other embryos. The correlation of particular maternal menstrual histories and the known developmental ages of monkey embryos allowed the construction of growth tables so that the size of an embryo (specifically the greatest length) could be used to predict its presumed age in postovulatory days. Streeter believed such estimations could be ± 1 day for any given stage. Within this staging system, which is more fully described for embryonic development on page 344, embryonic life commences with fertilization at stage 1, with stage 2 encompassing embryos from 2 cells, through compaction and early segregation to the appearance of the blastocoele (3.31^[3], 3.32^[4]).

The reader is also urged to examine 3.37^[5] which shows the developmental processes occurring between stages 1–10, and 4.1^[6] which shows the developmental staging used in the Embryology and Development section alongside the obstetric estimation of gestation used clinically.

Blastocyst

The blastocyst 'hatches' from its zona pellucida at 6–7 days, possibly assisted by an enzyme similar to trypsin. Trophoblast oozes out of a small slit, and many embryos form a figure 8 shape, bisected by the zona pellucida, especially if it has been hardened during oocyte maturation and cleavage. Such half-hatching could result in the formation of identical twins. Hatched blastocysts expand and differentiation of the inner cell mass proceeds.

The free unattached blastocyst is assigned to stage 3 of development (O'Rahilly & Muller 1987^[2]; see p. 140^[7]) at approximately 4 postovulatory days, whereas implantation (before villus development) occurs within a period of 7–12 days postovulation and over the next two stages of development. Two stage 3 blastocysts examined in some detail (Hertig et al 1954^[8]) include: one with 58 cells (3.33^[9]), of which only about 5 are inner cell mass and destined to form the embryo, the remainder being trophoblast, concerned with extraembryonic membranes and placentation; and another blastocyst consisting of 107 cells (3.34^[10]), of which 69 are mural trophoblast cells, 30 are polar trophoblast cells and 8 form the inner cell mass. Even at this early stage these 8 cells are already arranged into an upper layer (i.e. closest to the polar trophoblast), the *epiblast*, which will give rise to the embryonic cells, and a lower layer, the *hypoblast*, which has an extraembryonic fate. Thus the dorsoventral axis of the developing embryo and a bilaminar arrangement of the inner cell mass is established at or before implantation. (The earliest primordial germ cells may also be defined at this stage; Hertig 1968^[11].)

Implantation

On the sixth postovulatory day the blastocyst adheres to the uterine mucosa and the events leading to the specialized, intimate contact of trophoblast and endometrium commence (3.35, 36). Implantation is the term used for this complicated process; it includes the following stages:


- (1) dissolution of the zona pellucida
- (2) orientation and adhesion of the blastocyst onto the endometrium
- (3) trophoblastic penetration into the endometrium
- (4) migration of the blastocyst into the endometrium
- (5) spread and proliferation of the trophoblast, which envelops and specifically disrupts and invades the maternal tissues.

The embryo is drawn into a tight association with the uterine epithelium; uterine fluid is withdrawn by progesterone-sensitive pinopods, and short-range forces enable embryos to adhere to epithelia. The pentasaccharide lacto-N-fucopentose-1 on the epithelium and its receptor on the trophoblast could be specifically involved in adhesion. For details of placental development see page 166.


The trophoblast from stages 4 and 5 onwards has two distinct cell arrangements: *cytotrophoblast*, cuboidal cells which form the mural and polar trophoblast; and externally *syncytial trophoblast* (*syncytiotrophoblast*), a multinucleated mass of cytoplasm which forms initially in areas near the inner cell mass after apposition of the blastocyst to the uterine mucosa. It is the syncytial trophoblast which penetrates the uterine luminal epithelium. The uterine epithelial cell junctions are breached by flanges of syncytial trophoblast without apparent damage to the maternal cell membranes or disruption of the intercellular junctions; rather shared junctions are formed with many of the uterine epithelial cells (Enders et al 1983). As the blastocyst burrows more deeply into the endometrium syncytial trophoblast forms over the mural cytotrophoblast but never achieves the thickness of the syncytial trophoblast over the embryonic pole.

The youngest implanting human blastocyst recovered and described in detail (Hertig & Rock 1945, Carnegie embryo No. 8020 Stage 5a) shows an early stage in the process. The polar trophoblast displays an extensive syncytial development which projects into the endometrial stroma but syncytial lacunae have not yet developed. The blastocyst is not completely embedded and a portion of its wall at the abembryonic pole still projects into the uterine lumen. The age is believed to be 7 postovulatory days (Hertig & Rock 1945).

The *site of implantation* is normally in the endometrium of the posterior wall of the uterus, nearer to the fundus than to the cervix, and may be in the median plane or to one or other side.

Implantation may occur elsewhere in the uterus; implantation near the internal os results in the condition of *placenta praevia* with its attendant risk of severe antipartum haemorrhage (see p. 1873 ) , or in an *extrauterine* or *ectopic* site.

Ectopic Implantation


The conceptus may be arrested at any point during its migration through the uterine tube and implant in its wall. Previous tubule inflammatory episodes may predispose to such tubal arrest. It has been suggested that congenital abnormalities of the tube, tubal tumours, transperitoneal migration of a secondary oöcyte from one ovary to the opposite tube and delayed ovulation are additional predisposing factors of tubal implantation (Woodruff & Pauerstein 1969 .

Nidation of the intramural part of the tube often results in early abortion of the conceptus whereas, if it occurs elsewhere in the tube, development often proceeds for about 2 months and is then usually followed by tubal rupture with death of the embryo and severe intraperitoneal haemorrhage—a grave surgical emergency. However, slow rupture of the tube may occur, accompanied by a further implantation of the conceptus into any adjacent peritonealized surface (secondary abdominal pregnancy), which may lead to rupture of the surface with similar consequences.

Primary ovarian or *abdominal* pregnancies have also been described, in which it has been presumed that the fertilization occurred in the vicinity of the ovary; most cases, however, are probably of the secondary type following a slow tubal rupture or a slow extrusion of the conceptus through the abdominal ostium of the tube.

Apart from their important clinical implications, such conditions emphasize the interesting fact that the conceptus can implant successfully into tissues other than a normal progestational endometrium. Further, prolonged development can occur in such sites and is usually terminated by a mechanical or vascular accident and not by a fundamental nutritive or endocrine insufficiency or by an immune maternal response.

Postimplantation Development

The earliest developmental processes in mammalian embryos involve the production of the extraembryonic structures which will support and nourish the embryo during development. Production of these layers begins before implantation is complete. At present it is unclear where the extraembryonic cell lines arise. The trophoblast was considered to be a source but evidence now points to the inner cell mass as the site of origin. 3.37  shows the sequence of development of various tissues in the early embryo.

Amniotic Cavity

Ultrastructural examination of rhesus monkey embryos at the equivalent of stage 5a show that

the *epiblast* cells, which are closest to the implanting face of the trophoblast, have a definite polarity, being arranged in a radial manner with extensive junctions near the centre of the mass of cells, supported by supranuclear organelles (Enders et al 1986^[4]). A few epiblast cells are contiguous with cytotrophoblast cells; however, apart from this contact a basal lamina surrounds the now, initially, spherical cluster of epiblast cells isolating them from all other cells. Those epiblast cells adjacent to the hypoblast become taller and more columnar than those adjacent to the trophoblast; this causes the epiblast sphere to become flattened and the centre of the sphere to be shifted towards the polar trophoblast. Amniotic fluid accumulates at the eccentric centre of the now lenticular epiblast mass which is bordered by apical junctional complexes and microvilli. By day 10.5, in the rhesus monkey, there is a definitive *amniotic cavity* roofed by low cuboidal cells which possess irregular microvilli. The cells share short apical junctional complexes and associated desmosomes (Enders et al 1986^[4]) and rest on an underlying basal lamina. A demarcation between true amnion cells and those of the remaining definitive epiblast is clear. The columnar epiblast cells are arranged as a pseudostratified layer with microvilli, frequently a single cilium, clefted nuclei and large nucleoli; the cells have a distinct, continuous basal lamina. Cell division in the epiblast tends to occur near the apical surface, causing this region to become more crowded than the basal region. At the margins of the embryonic disc the amnion cells are contiguous with the epiblast; there is a gradation in cell size from columnar to low cuboidal within a two to three cell span (3.38^[5], 39^[6]).

Yolk Sac

The *hypoblast* just prior to implantation consists of a layer of squamous cells only slightly larger in extent than the epiblast. The cells exhibit polarity with apical microvilli facing the cavity of the blastocyst and apical junctional complexes, but they lack a basal lamina. During early implantation the hypoblast extends beyond the edges of the epiblast and can now be subdivided into those cells in contact with the epiblast basal lamina, the *visceral hypoblast*, and those cells in contact with the mural trophoblast, *parietal hypoblast*. The squamous parietal hypoblast cells may share adhesion junctions with the mural trophoblast and, rarely, gap junctions. The visceral hypoblast cells are cuboidal; they have a uniform apical surface towards the blastocyst cavity but irregular basal and lateral regions with flanges and projections underlying one another and extending into intercellular spaces. There is no basal lamina subjacent to the visceral hypoblast and the distance between the hypoblast cells and the epiblast basal lamina is variable.

A series of modifications of the original blastocystic cavity develops beneath the hypoblast later than those developing above the epiblast. Whilst the amniotic cavity is enlarging within the sphere of epiblast cells, the parietal hypoblast cells are proliferating and spreading along the mural trophoblast until they extend most of the way around the circumference of the blastocyst converging towards the abembryonic pole; at the same time a space appears between the parietal hypoblast and the mural trophoblast limiting the circumference of the hypoblastic cavity. A variety of terms have been applied to the parietal hypoblast layer: extraembryonic hypoblast and later extraembryonic endoderm or the exocoelomic (Heuser's) membrane. The cavity which the layer initially surrounds is termed the *primary yolk sac*, although O'Rahilly and Muller (1987^[7]) commend the term *primary umbilical vesicle*. There is considerable confusion concerning the developmental state of the primary yolk sac before initiation of the later cavity, the secondary

yolk sac, and in the way in which it forms (see Enders et al 1986^[1]). Enders et al conclude that the presence of a complete primary yolk sac cannot be determined from the material in the Carnegie collection or from their studies. The *secondary yolk sac* has been suggested to form in a variety of ways:

- from cavitation of visceral hypoblast (Hill 1932^[2]), a method similar to formation of the amnion
- rearrangement of proliferating visceral hypoblast (Heuser & Streeter 1941^[3])
- folding of the parietal layer of the primary yolk sac into the secondary yolk sac (Luckett 1978^[4]).

Certainly numerous mitotic figures are seen in the visceral hypoblast preceding secondary yolk sac formation, and at the margin of the visceral portion hypoblast cells overlie one another and appear to indicate a reflection of the layer. Enders et al (1986^[5]) conclude that a central portion of the secondary yolk sac may derive from parietal hypoblast and the remainder from visceral but point out that the derivation of the cells may be of little significance as experimental work has shown that in the mouse differentiation of parietal and visceral hypoblast can be reversed (Hogan & Tilly 1981^[6]).

The visceral hypoblast cells may later have a focus of production in the posterior margin of the disc. The cells later induce the formation of the primitive streak thus establishing the axis of the embryonic disc (Azar & Eyal-Giladi 1979^[7], 1981^[8]; Khaner & Eyal-Giladi 1989^[9]). With the later formation of the embryonic cell layers from the epiblast (see p. 142^[10]) the visceral hypoblast appears to be sequestered into the secondary yolk sac wall by the expansion of the newly formed embryonic endoderm beneath the epiblast (Tam & Beddington 1992^[11]).

It is necessary to point out at this juncture that the early embryonic bilaminar disc was thought to contain the outer layer—future skin—and inner layer—future gut lining—of the embryo, the ectoderm and endoderm. This was the basis of the germ layer theory. Experimental studies have now shown that all of the embryonic cell lines derive from the upper layer of the embryonic disc and that the lower layer has no embryonic fate. The terms epiblast and hypoblast are used to make this distinction between the earliest bilaminar disc layers and the later embryonic layers (see p. 92^[12] for a full account of the embryonic nomenclature).

Descriptions of several human blastocysts at stages 5 and 6 (O'Rahilly & Muller 1987^[13]) are available (3.36^[14], 38^[15], 39^[16]). The trophoblast is now divisible into cytotrophoblast and syncytial trophoblast over the whole of the blastocyst; it is thickest over the embryonic pole but diminishes in thickness over the sides and is exceedingly thin over the abembryonic pole, the last part to be embedded. The syncytial trophoblast which invades and becomes incorporated into maternal vessels encloses numerous lacunae containing maternal blood (Enders et al 1983^[17]).

Extraembryonic Tissues and Coelom

By definition extraembryonic tissues encompass all tissues that do not contribute directly to the future body of the definitive embryo, and later, the fetus. At stage 5 embryos are implanted but not yet villous; they range from 7–12 days old. A feature of this stage is the first formation of extraembryonic mesoblast which will come to cover the amnion, secondary yolk sac and the internal wall of the mural trophoblast and will form the connecting stalk of the embryo with its contained allanto-enteric diverticulum. The origin of this first mesoblastic extraembryonic layer is by no means clear. For many years it was thought to develop by delamination from the cytotrophoblast (Hertig & Rock 1949📖), although it was also suggested that it derived from the inner cell mass hypoblast (Heuser & Streeter 1941📖), or the caudal region of the epiblast (Luckett 1978📖). The fate of the first mesoblastic extraembryonic layer is at least two-fold and may in fact indicate more than one origin; it gives rise to both the layer known as extraembryonic *mesoblast*, arranged as a *mesothelium* with underlying *mesenchymal* cells; and also to *angioblastic tissue*, which forms the extraembryonic endothelia and blood cells. Recent investigators have disputed the idea of a trophoblastic origin of extraembryonic mesoblast after the observation that there is always a complete basal lamina underlying the trophoblast. The migration of cells out of an epithelium is usually associated with previous disruption of the basal lamina (Nichols 1985📖, 1986📖; Erickson 1986📖). The predominant layer of cells without a basal lamina in the blastocyst is the hypoblast. Enders and King (1988📖) have convincingly suggested that the earliest mesoblast derives from the parietal hypoblast which appears to form an extracellular structure corresponding to the *magma reticulare* between the mural trophoblast and the primary yolk sac in the stage 5 embryo. They have demonstrated the development of subhypoblastic cells into extraembryonic mesenchyme and into the earliest formed capillaries within developing villi of the placental disc. They note that it is not clear whether the extraembryonic mesenchyme derives from later proliferation of the earliest subhypoblastic cells or from continuous seeding from the hypoblastic layer, as both cell groups are mitotically active.

Epiblast cells are known to produce extraembryonic mesoblast prior to the development of the primitive streak. This mesenchymatous tissue initially mushrooms beneath the cytotrophoblastic cells at the embryonic pole forming the cores of the developing villus stems, and villi, and the capillaries within them (for information on placentation see p. 157📖, and for intraembryonic blood vessels see below).

At approximately 13 postovulatory days the conceptus consists of an outer cytotrophoblast (mural trophoblast), clothed externally by labyrinthine syncytiotrophoblast, and the beginnings of an extraembryonic mesoblastic lining; these layers together constitute the *chorion*. The cavity of the blastocyst is now termed the *chorionic cavity* (3.39👁). The embryo proper, situated to one side of the chorion, consists of an epiblastic layer of pseudostratified columnar cells resting on a basal lamina, which is contiguous with a simple layer of amnion cells. The amnion cells are in contact with extraembryonic mesoblast which separates them from the cytotrophoblast. Beneath the epiblast is a layer of visceral hypoblast which is reflected at the edges of the epiblast to form a small cavity, the secondary yolk sac; this may also contain some of the original parietal hypoblast within its wall. The chorionic cavity is filled with diffuse extracellular matrix and extraembryonic mesenchyme cells which attach to the secondary yolk sac wall to form a layer of extraembryonic mesenchyme covered with mesothelium. When the extraembryonic mesothelium has completely lined the mural trophoblast and covered both the amnion and the secondary yolk

sac the chorionic cavity can be termed the *extraembryonic coelom*.



Towards one end of the embryonic region the extraembryonic mesoblast surrounds a diverticulum of the visceral hypoblast, the allantois, which passes from the roof of the secondary yolk sac to the same plane as the amnion. Whereas initially extraembryonic mesoblast connects the amnion to the chorion over a wide area, with continued development and expansion of the extraembryonic coelom this attachment becomes increasingly circumvented to a *connecting stalk*, a permanent connection between the future *caudal end* of the embryonic disc and the chorion. The connecting stalk forms a pathway along which vascular anastomoses around the allantois establish communication with those of the chorion.


The conceptus at stage 5 consists of the walls of three cavities, amnion and yolk sac, connected at the embryonic bilaminar disc by the epithelial epiblast and visceral hypoblast, enclosed in a larger extraembryonic coelom (chorionic cavity). A fourth cavity, the *allantois*, will form as a hypoblastic diverticulum in stage 7. The 'bilaminar disc' commonly referred to in embryology texts does *not* yet possess the *definitive* embryonic ectoderm and endoderm layers which will give rise to embryonic structures. Only the epiblast will give rise to the embryo. All other layers produced so far are extraembryonic. The amnion and chorion (and surrounding mesoblast) are part of the *extraembryonic somatopleure* whereas the yolk sac, allantois and surrounding extraembryonic mesoblast constitute *extraembryonic splanchnopleure*. At the junctional zone surrounding the margins of the embryonic area, where the walls of the amnion and yolk sac converge, the somatopleuric and splanchnopleuric layers of extraembryonic mesoblast are continuous.

Formation of the Embryonic Tissues

At early stage 6 the epiblast is producing extraembryonic mesenchyme from its caudal margin. With the appearance of the *primitive streak* a process is begun whereby cells of the epiblast either pass deep to the epiblast layer to form the populations of cells within the embryo or they remain on the dorsal aspect of the embryo to become the embryonic ectoderm. The primitive streak marks the beginning of gastrulation, a period when gross alterations in morphology and complex rearrangements of cell populations occur. The epiblast will during this time give rise to a complex trilaminar structure with a defined craniocaudal axis. By the end of gastrulation, cell populations from different, often widely separated, regions of the embryonic disc will often become closely related and the embryonic shape will have been produced.




Primitive Streak


Seen from the dorsal (epiblastic) aspect, at stage 6, the embryonic disc appears elongated. The primitive streak is first seen in the caudal region of the embryonic disc, orientated along its long axis, conferring the future craniocaudal axis of the embryo (3.40 , 41 ). Although the future cranial and caudal regions of the embryo are well within the boundaries of the embryonic disc, it has become the practice to term the region of the disc closest to the streak caudal, and the region of the disc furthest from the streak cranial or rostral. With the development of the streak


the terms medial and lateral can now be used. The primitive streak is a midline proliferative region of the epiblast where cells may break free from the epithelium and migrate **beneath the epiblast** (3.41 ). At the cranial end of the streak there is a curved ridge of cells termed the primitive node (see below) and, in the midline of the node and streak, cells sink into a primitive groove prior to passing subjacent to the epiblast. *The relative dimensions of the primitive streak and the fates of the cells which pass through it change with the developmental stage.* Thus the streak extends half way along the disc in the stage 6 embryo, reaches its greatest relative length in stage 7 and its maximum length in stage 8. It is still present in stage 11 embryos but relatively few cells pass through it at this stage compared to the early stages.

The passage of epiblast cells through the primitive streak begins their transformation into all of the embryonic cell lines. In this way epiblast forms the embryonic endoderm, the notochord, the primordial germ cells and the mesoblast, as well as contributing extraembryonic mesoblast to the developing placenta. The epiblast cells which do not pass through the streak give rise to the neural and surface ectoderm of the embryo.

The primitive streak may be considered to be generally homologous with the blastopore of lower vertebrates (e.g. amphibia), with the nodal region corresponding to the dorsal lip. Experiments clearly show the lip of the blastopore to be a dynamic wave front on which cells are carried into the interior to form the roof of the archenteron, a situation analogous to ingression through the node of the prechordal plate and endoderm.

The primitive streak similarly may be considered analogous to the coapted, or fused, lateral lips of the blastopore. Finally, the cloacal membrane and its immediate environs are considered analogous to the ventral lip of the blastopore. This homology is strengthened by studies which suggest that both the primitive node and the primitive streak represent the 'organiser' of the amniote embryo (Tam & Beddington 1992 ). The dorsal lip of the blastopore was known many years ago to act as an organizer in amphibian embryos (Spemann & Mangold 1924 ). The experiments which have now demonstrated a homeobox gene, goosecoid, in *Xenopus* prior to dorsal lip formation, in the same position as Spemann's organizer, and have further demonstrated goosecoid expression in the cephalic end of the mouse primitive streak confirm this homology (De Robertis et al 1992 .

At the primitive streak, epiblast cells undergo a period of intense proliferation, the rate of division being much faster than that of blastomeres during cleavage (Graham 1973 ). Streak formation is associated with:

- the local production of several cell layers
- extensive disruption of the basal lamina
- increase in adhesive plaques and gap junctions
- synthesis of vimentin and loss of cytokeratins by the emerging cells (Lawson et al 1991 .

The process by which cells become part of the streak and then migrate away from it is termed *ingression*; it relies on a complete layer of visceral hypoblast beneath the epiblast (Bellairs 1987) as well as loss of the epiblast basal lamina at the region of the streak. Azar and Eyal-Giladi (1979, 1981) have shown that the hypoblast induces the formation of the primitive streak. Further it has been demonstrated, in the chick, that the original caudal marginal zone, where the hypoblast cells are generated, prevents other regions of the hypoblast from forming hypoblastic cells and thereby other primitive streaks (Khaner & Eyal-Giladi 1989). Tam and Beddington (1992) provide other evidence to support this finding, noting that the hypoblast beneath the streak does not seem to be replaced by the embryonic endoderm, which sequesters the visceral hypoblast into the secondary yolk sac (see below), even at late streak stages.

Primitive Node

Primitive node, or Hensen's node is the most rostral region of the primitive streak. It appears as a curved ridge of cells similar in shape to the top of an old-fashioned keyhole. Cells ingressing from the ridge pass into the primitive pit (the most rostral part of the primitive groove) and then migrate rostrally beneath the epiblast. The primitive node has been recorded in all stage 7 human embryos. At this time, early to midstreak stages, the primitive streak achieves its greatest relative length, about 50% of the total length of the embryonic disc. The primitive node produces axial cell populations, the prechordal plate, notochord, embryonic endoderm and the medial halves of the somites.

Several workers have noted that in the chick, the node can induce the formation of an extra axis when grafted to a host embryo (Selleck & Stern 1992; Schoenwolf et al 1992), and further it can induce supernumerary digits when grafted into the anterior margin of developing limb buds, properties not seen in other regions of the epiblast. Removal of the node results in complete absence of the notochord and a loss of control of neurulation (O'Rahilly & Muller 1986).


Fate Maps

Maps of the epiblast at the primitive streak stage have been derived for many vertebrates. They indicate the putative cell lines which derive from the epiblast layer and suggest that most, perhaps all, chordate embryos share a common strategy of early tissue allocation. The first fate maps of mammalian epiblast (Beddington 1981, 1982; Tam & Beddington 1987) illustrate the broad similarity in composition of the epiblast in a range of vertebrates. Studies of cell fate have shown that epiblast cells which will pass through the streak can be identified, randomly located within the epiblast layer, before their ingression (Stern & Canning 1990); that epiblast fate is determined at or before the time of ingression through the streak, suggesting that *passage through the streak* is the most important factor for future differentiation; and that the position of ingression, be it through the streak or node, directly affects the developmental fate of the cell (Lawson et al 1991). For a composite of the information on the position of ingression through the streak and node see 3.42.






Time of Ingression



The time at which epiblast cells pass through the streak will affect the future fate of the cells. There is an enormous rate of growth of the embryonic disc during the primitive streak stage. In the mouse the embryonic axis increases 3.5 fold in length between the prestreak and neural plate stages. At each stage of development the streak is slightly different, as is the embryo, therefore descriptions of streak stage embryos, and of cells ingressing through the streak must specify the stage of streak development. Often the primitive streak stage is subdivided into early, mid or late streak stages.

Position of Ingression

Position of ingression through the node or streak. This is also important in deciding the fate of particular cells. Passage through the streak is specified according to position, e.g. via the node, rostral, middle or caudal regions of the streak. Cells ingressing through the primitive node give rise to the axial cell lines, endoderm, notochord and the medial halves of the somites. The rostral portion of the primitive streak produces cells for the lateral halves of the somites, whereas the middle streak produces the lateral plate mesoblast. The next caudal portion of the streak gives rise to the primordial germ cells, which can be distinguished histologically and histochemically at midstreak stages, and the most caudal portion of the streak contributes cells to the extraembryonic mesoblast until the early somite stage. It is notable that most of the cells termed mesoblast, produced by ingression through the middle and rostral streak regions, differentiate into epithelia on reaching their initial destination (Bellairs 1987.

Embryonic Endoderm

The earliest cells migrating through the primitive node and streak give rise to both the *embryonic endoderm* and the *notochord*. Definitive embryonic endoderm has been shown to be derived from epiblast cells located at the primitive node and rostral primitive streak by fate mapping and in situ labelling studies (Lawson et al 1991; Selleck & Stern 1991). It has been demonstrated, in the mouse, that by the midstreak stage the endodermal cells are beneath the epiblast mainly in the midline, interspersed with presumptive notochordal cells, forming the roof of the secondary yolk sac (Tam & Beddington 1992). The ingressing endoderm is suggested to displace the visceral hypoblast into the secondary yolk sac wall by a dramatic territorial expansion, probably brought about by a change in the morphology of these epithelial cells. The putative endoderm cells are cuboidal within the node and become squamous in the endoderm layer; this could result in a fourfold increase in the surface area covered by the cell population (Tam & Beddington 1992). However, a complete replacement of the visceral hypoblast has not yet been confirmed and there may be a mixed population of cells in the endodermal layer in the early stages. For the developmental fate of the embryonic endoderm see 3.43.

Ingression of cells through the streak and node in the human is apparent at stage 6, and by stage 7 a population of endoderm and notochord cells is present beneath the epiblast (3.41, 43). From stages 6–11 the roof of the secondary yolk sac is formed by a midline population of

cells, the notochordal plate, in direct lateral continuity with the endodermal cells. It is not until stage 11 that the definitive notochord is formed in the pharyngeal region and the endoderm cells can join across the midline.

Prechordal Plate

The prechordal plate is defined as a localized thickening of the endoderm rostral to the notochordal process. As such it represents the first population of endoderm cells to ingress through the primitive node. There is some confusion over the limits and fate of the prechordal plate (sometimes prochordal plate). The term has been used to describe:

- an area of endoderm
- the buccopharyngeal membrane
- an accumulation of mesenchymal cells immediately rostral to the notochord believed to be derived from the endoderm (Adelmann 1922^[1], 1926^[2]), a view which has been supported until quite recently (Noden 1988^[3]).

The majority of the endodermal and notochordal cells become epithelial after ingression, the endoderm forming the roof of the secondary yolk sac with a local, prechordal region up to 8 cells thick, while the notochordal cells form an epithelial rod between the epiblast and endoderm. (The epithelial prechordal plate forms the endodermal layer of the buccopharyngeal membrane, the pre-oral gut and probably all of the foregut.) A small group of cells, however, remain mesenchymal rostral to the notochord and beneath the epithelial endoderm; these form the most cranial axial mesenchyme population and they are termed *prechordal mesenchyme*. Wachtler and Jacob (1986^[4]) have demonstrated that the notochordal process is composed of determined myogenic cells immediately after its formation. The posterior portion becomes transformed into the chorda (notochord proper) whereas the prechordal cells retain their mesenchymal morphology and myogenic fate. Orthotopic grafting has demonstrated that cells leave the edges of the prechordal mesenchyme and migrate laterally into the periocular mesenchyme; they give rise to all the extrinsic ocular muscles (p. 154^[5]) (see also p. 274^[6]).

Notochordal Process

The notochordal process in the stage 8 embryo can be described in three parts:

- a rostral part composed of a cell mass continuous with the prechordal mesenchyme
- a mid portion with cells arranged in a tube with a central notochordal canal
- a caudal portion which consists of a U-shaped arrangement of cells, the notochordal plate, contiguous with the embryonic endoderm.

In the latter arrangement the floor of the notochordal canal has broken down opening the notochordal canal to the secondary yolk sac. This groove and its connection to the primitive node

is termed the neurenteric canal. The notochordal process has also been termed the head process, the chordamesoderm, or chorda. It has been suggested that the ingression of notochordal cells is matched by specification of overlying neural ectodermal cells and that both cell lines arise from a common progenitor cell. The notochordal plate is thus matched in length by the future neural floor plate (Jessell et al 1989; see also p. 146).

Notochordal Plate

The notochordal plate roofs the secondary yolk sac and early gut until stage 11, at which time the pharyngeal region is the last to contain notochordal plate, the remainder of the plate having formed definitive notochord. The definitive notochord separates from the alimentary tract by a mechanism similar to formation of the neural tube (see p. 217). The most caudal part of the notochordal plate is in continuity with the primitive node, and here, as noted, there may be a transient connection between the amniotic cavity and the secondary yolk sac, the neurenteric canal. (The latter is so named because its upper opening is in the future caudal floor of the neural groove; its lower opening is into the archenteron. See also p. 98.)

Intraembryonic Mesoblast (Mesenchyme)

The morphology of cells passing through the primitive streak is well documented (Sanders 1986). Epiblast cells ingress through the cranial and middle parts of the streak individually, maintaining their apical epithelial contacts while elongating ventrally. The cells become flask-shaped with thin attenuated apical necks and broad basal regions. The basal and lateral surfaces form lamellipodia and filopodia and the apical contact is released. The cells are now free *mesoblast* cells, their fibroblastic, stellate morphology reflecting the release from the epithelial layer. Once through the streak the cells migrate away using as a substratum the basal lamina of the overlying epiblast and extracellular matrix. The cells contact one another by filopodia and lamellipodia, with which they also contact the basal lamina. Gap junctions have been observed between filopodia and cell bodies. With the appearance of the mesoblast, spaces form between the epiblast and visceral hypoblast which are filled with extracellular matrix rich in glycosaminoglycans. The migrating mesoblast has a leading edge of cells which open up the migration routes and the following cells seem to be pulled along behind in a coordinated mass movement.

Details of the embryological terminology can be found on page 93; however, it should be noted that the terms mesoblast and mesenchyme are being used in a specific manner and are not interchangeable. Previously, cells forming a population between the epiblast and hypoblast were termed mesoderm, or, more recently, mesenchyme. Hay (1968) commended the terms primary and secondary mesenchyme to distinguish between those cells arising from ingression through the streak and from neural crest ingression (see also p. 93). The primary mesenchymal cells will revert to epithelia at their destinations. However, whereas some primary mesenchymal cells may become epithelial within a short time frame, for example somites and lateral plate, other cells may differentiate into endothelium much later, or change to endothelium then back to mesenchyme once more. To cope with these conflicts in terminology the mixed population of epiblast cells which ingress through the primitive streak and come to lie between the epiblast and

embryonic endoderm will be termed mesoblast until their fate as specific mesenchymal or epithelial populations is clear.

Primordial Germ Cells

Although early studies on human embryos have reported primordial germ cells and described their development from the early endoderm of the yolk sac and allantois it is now clear from animal experimentation that the primordial germ cells arise from epiblast ingressing at the caudal end of the primitive streak. Where these epiblast cells are located on the embryonic disc, i.e. from rostral regions which migrate to the streak or from local caudal regions, has not been elucidated. However, some studies have demonstrated *extremely early segregation* of the germ cells, when the epiblast layer consists of only 10–13 cells (Soriano & Jaenisch 1986¹). It is suggested that the primordial germ cells remain sequestered in the extraembryonic mesenchyme at the caudal end of the embryo until the embryonic endoderm has been produced and gastrulation completed (Ginsburg et al 1990²), then with the folding of the embryo underway, the primordial germ cells begin their migration along the allantoic and hind gut endoderm. The formation of the tail fold brings the proximal portion of the allantois within the body reducing the final distance over which the cells migrate to the genital ridges.

Embryonic Ectoderm


When the ingression of cells through the primitive streak is completed the cells remaining in the epiblast layer are termed embryonic ectoderm cells. This layer still contains a mixed population as both surface ectoderm cells and neural ectoderm cells are present. It is suggested that these cells were originally in the cranial half of the disc at the early streak stage, with the neural fated cells being closest to the streak and the surface ectoderm being most cranial (3.42³). The process of neurulation resites most of the neuroepithelial cells (see below). For the developmental fates of the surface and neural ectoderm see 3.43⁴.

Trilaminar Disc

The stage 8 embryo, of approximately 18–19 postovulatory days, has three layers present and can be termed the trilaminar disc. It is pear-shaped, broader cranially than caudally. The upper *epiblast* cells are tall, forming a pseudostratified columnar epithelial layer with a basal lamina, except at the primitive streak where the cells are ingressing to form the other layers. The more cranially placed epiblast will give rise to the surface ectoderm. The extent of the future neural plate can be assessed; it is correlated to the length and width of the notochordal plate directly beneath. The lower embryonic endoderm, a simple squamous layer with a developing basal lamina, is not always complete at this stage particularly in the midline caudal to the prechordal plate, which is still occupied by the notochordal process or plate.



The middle, mesoblast, layer is composed of free cells migrating cranially, laterally and caudally from the primitive streak. They produce extracellular matrix which separates the epiblast and endoderm of the embryonic area permitting their passage. The streams of mesoblast which pass

in a cranial direction flank the notochordal plate, pass around the prechordal plate region and then converge medially to fuse in the midline beyond its cephalic border. This transmedian mass is the cardiogenic mesoblast in which the heart and pericardium are to develop. Around the extreme cephalic margin of the embryonic area, the cardiogenic mesoblast fuses with the junctional zone of extraembryonic mesoblast. This region will eventually form the septum transversum and primitive ventral mesentery of the foregut. Mesoblast passing laterally from the streak soon approaches and becomes confluent with the extraembryonic mesoblast around the margins of the disc, i.e. at the *junctional zone* where the splanchnic and somatic strata of extraembryonic mesoblast merge.

The mesoblast which streams caudally from the primitive streak skirts the margins of the cloacal membrane (see below) and then converges towards the caudal midline extremity of the embryonic disc to become continuous with the extraembryonic mesoblast of the connecting stalk. Thus the mesoblast extends between the epiblast and endoderm over all of the disc area except cranially at the prechordal plate (see p. 144 ) , a portion of which will become the *buccopharyngeal membrane*, and caudally at the *cloacal membrane*. The cloacal membrane is a patch of thickened endoderm, similar to the prochordal plate, caudal to the primitive streak. At the present time it is not clear if the lower layer of the cloacal membrane consists of visceral hypoblast like the more cranial primitive streak, (the hypoblast is necessary for maintaining the streak), or if it is replaced by migrating embryonic endoderm, or if there is a region for ingression of endoderm at the caudal end of the streak similar to the node cranially.




Still further caudally the embryonic disc develops a midline diverticulum adjacent to the cloacal membrane, this diverticulum, the *allantois*, projects into the extraembryonic connecting stalk. There is little information concerning which cells form the allantois, i.e. whether it is composed of visceral hypoblast, parietal hypoblast or embryonic endoderm. The allantois later develops a rich anastomotic blood supply around it in the manner of the yolk sac.

Neurulation

Neurulation begins at stage 9 (3.44  , 45 ). The process, although continuous spatially and temporally, can be divided into four stages:

- (1) local elongation of the ectoderm cells in a midline zone of the disc and their reorganization into a pseudostratified epithelium, the *neural plate*.
- (2) reshaping of the neural plate.
- (3) bending of the plate into a *neural groove*.
- (4) closure of the neural groove into a *neural tube* from the midportion to its cranial and caudal ends with formation of a continuous surface ectoderm dorsal to the tube.

The regions of rostral and caudal fusion are termed rostral and caudal neuropores respectively.

The extent of the neural plate initially corresponds precisely in length to the underlying notochord and axial tissues; thus it extends from the cranial border of the primitive node to the buccopharyngeal membrane (3.46 ). Later it extends laterally beyond the notochord to cover the paraxial mesenchyme. Studies have suggested that the neural floor plate cells arise from a common progenitor cell which also provides the cell line for the notochord (Jessell et al 1989 ) (see also p. 226 .


During the period of neurulation extensive changes are taking place in the embryonic mesoblast. Mesoblast cells migrate to their destinations, predetermined by their passage through the streak or node, and rapidly show differences in morphology and organization. Initially, two separate cell populations can be identified:

- a thickened medial portion which lies close to the notochord and elevating neural folds, the *paraxial mesenchyme*
- a flattened lateral portion which extends to the periphery of the embryonic area, the *lateral plate mesenchyme*; this mesenchyme is contiguous with the extraembryonic mesoderm which covers the amnion and yolk sac walls.

The paraxial mesenchyme shows the earliest differentiation. When the neural folds fuse, initially at the level of the hindbrain and cervical cord, the paraxial mesenchyme forms segmental condensations each side of the neural tube, the epithelial somites. Between 4 and 12 pairs of somites are formed during stage 10, the most caudal somites being on a level with the caudal neuropore; they develop caudally from unsegmented mesenchyme at a rate which matches the caudal fusion of the neural tube (see somites below).



As the neural plate grows, its margins become raised as the *neural folds*. The neural folds become particularly prominent at the cranial end of the disc, the future forebrain. Here the folds are separated rostrally from each other by the terminal notch, which abuts the buccopharyngeal membrane. Three major divisions of the brain appear at this time, before the neural tube has formed. They are marked by two slight transverse constrictions on the surface ectoderm indicating a division into the prosencephalon or forebrain, the mesencephalon or midbrain and the rhombencephalon or hindbrain.

Neural Tube Formation






This is closely linked to changes in cell shape. The neural ectodermal cells become elongated and then wedge shaped. It has been suggested that the forces needed to shape the neural tube are intrinsic to the neur ectoderm cells themselves; neurulation occurs as a result of the changes in shape of these cells generated by their cytoskeleton elements (Schoenwolf & Smith 1990 ). The entire neural plate is also elongating and has considerable cell proliferation at this time. The lateral mesenchymal cells and extracellular matrix provide support for the elevating folds and surface ectoderm and aid the alignment of the neural layers at fusion.

The neural groove deepens, its lateral then dorsal edges come into contact and fuse to convert the groove into a sagittal slit-like canal. The overlying surface ectodermal layers from each side likewise fuse over the neural tube. Neural tube fusion occurs first in the hindbrain or upper cervical region in the third week (stage 10, 22–23 postovulatory days). The fusion extends both rostrally and caudally until only a small opening is left at each end. These are the rostral and caudal neuropores; they close in the middle and latter ends of the fourth week respectively. The neural tube forms the central nervous system (CNS). After closure of the neural tube has commenced, ridges can be seen on the floor of the rhombencephalon: these are termed *rhombomeres* and they have significance for patterning of the brain and head region (for a more detailed account of neurulation see below).

Neural Crest

Neural crest is the name given to a band of cells at the outermost edges of the neural plate, adjacent to the presumptive epidermis (3.46 ) . Neural crest cells, from the head region, assume a mesenchymal morphology and begin migration prior to neural tube closure; in the trunk, the cells remove themselves from the epithelium as the neural tube closes. They lie on the dorsal part of the newly formed neural tube for some time before migration. Crest cells can migrate over considerable distances, they contribute a major population of mesenchyme to the head, and also to a wide range of different cell lines in the trunk. They are referred to either as neural crest cells or as ectomesenchyme, to note their derivation from the ectoderm. (Hay 1968 ) commended the term secondary mesenchyme.) They do not usually give rise to epithelial tissues. They give rise to much of the peripheral nervous system (PNS) (see below).


Ectodermal Placodes


These make a significant contribution to the PNS and are intimately involved in the formation of the cranial sensory ganglia (see p. 224 ) . After the neural crest cells have begun their migration and have passed beneath the placodal regions the surface ectoderm shows localized thickenings. In the lateral regions of the pharyngeal arches, at stage 10–11, cells remove themselves individually from the ectoderm and become associated with neural crest cells in the cranial sensory ganglia supplying these arches (see p. 224 ) . Larger ectodermal placodes retain their epithelial nature and become closely associated with the neural tube. Two otic placodes are localized one on each side at the lateral region of the second pharyngeal arch; they give rise to the fluid filled labyrinth of the inner ear (see p. 262 ) . One midline placode invaginates as the adenohypophysis (see p. 257 ) immediately rostral to the buccopharyngeal membrane. Prior to neurulation, the cells which will give rise to the adenohypophyseal placode lie in the midline, rostral neural fold (neural ridge) (3.100 ) . There are a variety of non-neural placodes. The paired optic placodes give rise to the lens in each eye; similar specializations of the ectoderm in the head give rise to the outer coating of the teeth.

Folding of the Embryo


In a diagrammatic representation of the disc viewed from the ectodermal aspect, all of the future

external surface of the body is delimited. Each end of the gut tube is specified on the ectodermal surface at the buccopharyngeal and cloacal membranes, regions where the ectoderm and underlying endoderm are opposed without intervening mesoblast. In the midline between these membranes the neural tube will form from fusion of the lateral edges of the neural plate and the surface ectoderm will fuse in the midline to constitute the future skin of the back.

The representation of a person on the trilaminar disc (3.47 ) shows to some extent the way in which the positions of the main body structures are already specified in the unfolded embryo. The portion of the disc between the buccopharyngeal membrane and the edge of the disc will become the anterior thoracic wall and the anterior abdominal wall cranial to the umbilicus. Further caudally, midway along the neural axis, the lateral portions of the disc will become the lateral and anterior abdominal walls of the trunk; that portion of the disc beyond the cloacal membrane will form the anterior abdominal wall caudal to the umbilicus. Thus the circumference of the disc, where the embryonic tissue meets the extraembryonic membranes, will become restricted to the connection between the anterior abdominal wall and the umbilical cord, i.e. the umbilicus.


Head folding begins at stage 9 when the fusing cranial neural plate rises above the surface ectoderm and the portion of the disc rostral to the buccopharyngeal membrane, containing the cardiogenic mesenchyme, moves to lie ventral to the developing brain. The prosencephalon and buccopharyngeal membrane are now the most rostral structures of the embryo. The previously flat region of endoderm, the prechordal plate, is now modified into a deep tube, the primitive foregut. Tail folding can be seen in stage 10 embryos when the whole embryo comes to rise above the level of the yolk sac. The similar movement of the part of the disc caudal to the cloacal membrane results in its repositioning ventral to the neural plate. Generally, as the embryo rises above the edges of the disc the lateral regions of the disc are drawn ventrally and medially, contributing to the lateral folding of the embryo. (For a full understanding of this process it is necessary to study the diagrams in 3.48A-G )

Formation of the Intraembryonic Coelom




At and just before stage 9 (before formation of the head fold), vesicles appear between the mesenchymal cells cranial to the buccopharyngeal membrane and within the cranial lateral plate mesenchyme. At the periphery of the vesicles the mesenchymal cells develop junctional complexes and apical polarity, thus forming an epithelium. The vesicles become confluent forming a horseshoe-shaped tube, the *intraembryonic coelom*, which extends caudally to the level of the first somite and laterally into the lateral plate mesenchyme towards the extraembryonic mesenchyme; the intra- and extraembryonic coeloms do not communicate at this stage. The lateral plate mesenchyme thus develops somatopleuric coelomic epithelium subjacent to the ectoderm, and a splanchnopleuric coelomic epithelium next to the embryonic endoderm (3.49 )

During development of the head fold the morphological movements which organize the foregut and buccopharyngeal membrane have a similarly profound effect on the shape of the

intraembryonic coelom. The midline portion of the originally flat, horseshoe-shaped coelom moves ventrally leaving the caudal arms of the horseshoe in their original position. Thus the midline part of the coelom, which was originally just rostral to the buccopharyngeal membrane, comes to lie anterior (ventral) to the foregut (caudal to the buccopharyngeal membrane), and the two lateral extensions of the coelom pass close to the lateral walls of the foregut on each side: the caudal portions of the coelom (the two arms of the horseshoe), which in the unfolded disc communicated laterally with the extraembryonic coelom, turn 90° to lie lateral to the gut, communicating with the extraembryonic coelom ventrally.

Compartments of the coelom which will later in development give rise to the body cavities can already be seen; the midline ventral portion, caudal to the buccopharyngeal membrane, becomes the pericardial cavity, the canals lateral to the foregut (pericardioperitoneal canals) become the pleural cavities and the uppermost part of the peritoneal cavity, and the remaining portion of the coelom becomes the peritoneal cavity. By stage 11 the intraembryonic coelom within the lateral plate mesenchyme extends caudally to the level of the caudal wall of the yolk sac. The intra- and extraembryonic coeloms communicate widely each side of the midgut along the length of the embryo from the level of the 4th somite (3.50 ) .

In the early embryo the intraembryonic coelom provides a route for the circulation of coelomic fluid which, with the beating of the heart tube, functions as a primitive circulation, taking nutritive fluid deep into the embryo, until superseded by the blood vascular system.

In spite of the importance of the coelom in defining the body cavities, and of the coelomic epithelium in the production of the major mesenchymal populations of the trunk (3.51 ) , only a few workers have considered the overall contribution of the coelom and its epithelium to the embryo (Streeter 1942 ; Langemeijer 1976 ). The coelom can be described as a single, tubular organ comparable to the neural tube in that it possesses a specialized wall that encloses a cavity. Certainly the proliferating coelomic epithelium has many similarities to the neural ectoderm. It is pseudostratified columnar epithelium with an inner germinal layer from which cellular progeny migrate. Both epithelia after the germinal phase ultimately form the lining of a cavity, ependyma for the neural epithelium and mesothelium for the coelomic epithelium.


The coelomic epithelium like the neural epithelium produces cells destined for different fates from different sites and developmental times. It is noticeable that the coelomic cells (like the neural epithelium) have apical epithelial specializations but tapering processes below which directly contact the underlying mesenchyme, there being no basal lamina. The possibility of the tapering processes forming directional signals for migrating progeny, similar to radial glia of the neural tube, has not been examined.

The proliferating coelomic epithelium produces two types of progeny: mesenchymal cells, either general or localized populations, and epithelial cells. The mesenchymal cells may become epithelial as their development fate; the epithelial derivatives, however, retain their epithelial differentiation.


General mesenchyme is produced from nearly all of the coelomic epithelium; it gives rise to the smooth muscle and connective tissue coats of tubes, e.g. of the gut, respiratory tract, reproductive and urinary tracts. Localized mesenchymal populations give rise to, among other structures: the septum transversum from the caudal pericardial region (forms part of the liver); a proliferation from the splanchnopleuric coelomic epithelium of the pleuroperitoneal canals dorsal to the stomach (forms part of the spleen); intermediate mesenchyme from the junction of the splanchnopleuric and somatopleuric coelomic epithelia (forms part of the early embryonic kidney).


Specific regions of the coelomic epithelium produce epithelial progeny which differentiate into structures as diverse as cardiac muscle, podocytes of the pronephric kidney (particularly in fish, avian and reptilian embryos), mesonephric epithelia, sustentacular cells which surround the primordial germ cells, and the epithelia lining the male and female reproductive tracts.

The coelomic channel and the primitive circulation which passes through it is of paramount importance up to stage 13. Whereas the superficial tissues of the embryo can receive nutrients via the amniotic sac and yolk sac fluids, the deeper tissues are, until the formation of the coelom, under conditions similar to tissue culture. From stage 10 however, exocoelomic fluid, propelled by the first contractions of the developing heart, is brought into contact with the deeply placed mesenchyme. This early 'circulation' ensures an adequate supply of nutrients to the rapidly increasing amount of embryonic tissue, and meets most of the requirements of the deeper mesenchymal derivatives.

From stage 12 the endothelial system expands, filling rapidly with plasma which passes across the locally thinned coelomic epithelium into the large hepatocardiac channels which project into the pericardioperitoneal canals at the level of the 7th somite (O'Rahilly & Muller 1987.

With the formation of the coelomic epithelium and the intraembryonic coelom the stage of gastrulation is over and organogenesis is underway. Just as the very early bilaminar disc could be viewed as an arrangement of three cavities (plus the allantois), now the same cavities can be examined after folding and the **embryonic** layers continuous with the walls of those cavities can be specified:


- the ectoderm and amnion line the amniotic cavity
- the embryonic endoderm and visceral endoderm line the yolk sac and allantois
- the intra- and extraembryonic coelomic epithelium lines the coelomic cavity, which consists of a large extraembryonic coelom continuous with a smaller intraembryonic coelom (3.52.


Each of these epithelia is supported by mesenchyme; all of the organs of the body will develop from interactions between one of these epithelial layers and its underlying mesenchyme. For an overview of the fate of the embryonic tissues see 3.43.

Embryonic Tissues


The cells of the embryo after gastrulation are arranged in two fundamental types of tissue, epithelial and mesenchymous and, despite regional modifications introduced as the various tissues develop, these types persist in large measure throughout life.


Epithelial Tissues

These are tissues in which the cells are closely packed, with narrow intercellular clefts containing minimal extracellular material, and a developed basal lamina containing specific proteins synthesized by the epithelium itself. The cells usually show juxtaluminal intercell surface specializations such as desmosomes, tight junctions, gap junctions, etc. (pp. 27–29) and specializations of the apical surface, which may exhibit microvilli or cilia. Characteristically epithelia clothe internal and external surfaces as simple or compound cellular sheets which separate phases of differing composition (e.g. the external environment and the subepithelial tissue fluids, intravascular and extravascular fluids, etc.). Traffic of materials in the intercellular clefts between cells is limited and passage occurs across the cells and their limiting membranes, which function as energy-dependent selective barriers, enhancing the passage of some materials and impeding the passage of others.



Historically the embryo was considered to be at the onset of organogenesis once three layers had been formed (see p. 92). However in the stage 11 embryo there are many more than three separate epithelial populations; they include surface ectoderm, the neural tube, notochord, embryonic endoderm, somites, somatopleuric and splanchnopleuric coelomic epithelia, epithelial ducts from intermediate mesenchyme and a range of endothelia. The majority of the derivatives of these layers retain their epithelial character throughout life; local invaginations produce glands and duct linings, which may retain their connection with their parent epithelia, although they may become detached as in the case of endocrine glands. Throughout development all epithelial tissues require underlying cells, sometimes in the form of other epithelial layers but more likely as free cells collectively termed mesenchyme to support them and engage in developmental processes.

Mesenchyme



This is a term first introduced over a century ago (Hertwig 1881) as an alternative to mesoblast. Mesenchyme cells have no polarity; they form junctional complexes which are not exclusively juxtaluminal and they produce extracellular matrix molecules and fibres from the whole cell surface. Mesenchymal populations form most of the tissue of the early embryo, occupying all the regions between the various epithelial layers described above.

Mesenchymal cells support epithelia throughout the developing body, both locally where they contribute to the basal lamina and smooth muscle of tubes, and generally where they differentiate into connective tissue (see p. 110). Specific mesenchymal populations control the patterning


of local regions of epithelium.






The space beneath epithelia and between mesenchyme cells is filled with *extracellular matrix molecules* and *fibres*; these include localized molecules of the basal lamina (e.g. laminin, fibronectin, etc.) and much larger complex associations of collagen, glycosaminoglycans, proteoglycans and glycoproteins between the mesenchyme cells. The external shape changes of the embryo reflect different rates of production of matrix molecules which support mesenchyme cells and their overlying epithelia; these molecules also provide migration routes for mesenchymal populations, and further, organize migrating cells by aligning intracellular proteins according to depositions of specific matrix molecules. Many of the signals for migration or differentiation are received from cells via matrix molecules, be they in the basal lamina, or in the matrix between cells. The presence or absence of certain matrix molecules may cause mesenchyme cells to migrate, or to stop migration and commence differentiation (see pp. 111 , 112 )

Subdivisions of the Mesenchyme

The first mesoblast population of the trilaminar disc (termed primary mesenchyme by Hay (1968 )) derives from epiblast cell ingression at the primitive node and streak. On reaching its final destination this mesoblast can be subdivided, by position, into populations of mesenchyme cells, i.e. *axial*, *paraxial* and *lateral plate* (3.51A ) . Local proliferation of these mesenchymal populations produces the enormous growth and expansion of the embryo. *Intermediate* mesenchyme develops slightly later between the paraxial mesenchyme and the lateral plate. A later, secondary, contribution of mesenchyme comes from neuroectoderm cells, i.e. the *neural crest*. Although the fate of much of the trunk neural crest is to form parts of the PNS, in the head crest cells contribute a significant population of ectomesenchyme which specifies the pattern of development in the viscerocranium.

Axial Mesenchyme

The first epiblast cells to ingress through the primitive streak form the endoderm and notochord. These cells initially occupy a midline position with the earliest endodermal cells forming the prechordal plate, but later the notochordal cells remain medially and the endodermal cells flatten and spread laterally. A population of cells which remain mesenchymal just rostral to the notochordal plate are termed *prechordal mesenchyme* (3.148 ) . These axial mesenchyme cells are tightly packed, unlike the more lateral paraxial cells, but are not contained in an extracellular sheath as is the notochord.

Adelmann (1927 ) described the development of the oculomotor muscles in the chick from mesenchyme formed from the prechordal plate. This was in conflict with later studies which suggested that extraocular muscles arose from small cavities in the mesenchyme each side of the diencephalon in mammals, the so-called preotic somites (Gilbert 1952 , 1957 )—a view supported by Meier (1984 ) and Noden (1983a ) who suggested that the most cranial somitomeres (see below) develop into the extrinsic ocular muscles. However Adelmann's

hypothesis has been confirmed by chimera experiments (Christ et al 1986; Wachtler & Jacob 1986), which showed that prechordal mesenchyme is displaced laterally at the time of head flexion and that the cells become integrated with those of the paraxial mesenchyme. Later this mesenchyme migrates to occupy the wall of the local head cysts where it provides the precursor cells for all of the extrinsic ocular muscles.

Notochord

Also called the chordamesoderm, this arises from epiblast cells of the medial part of the primitive node (Selleck & Stern 1991). It passes through several stages during development. The earliest notochordal cells are termed the notochordal process, or head process; they are intimately mixed with the endodermal cells. A canal has been identified in the notochordal process but seems not to be present in the notochordal plate, which remains from stage 8 to stage 11.

The development of the notochordal plate into notochord proceeds longitudinally from caudal to rostral, and the last areas to retain the plate are in the pharynx. The mechanism of notochordal formation is similar to, but a mirror image of, that of neurulation. The notochordal plate forms a deep groove; the vertical edges of the groove move medially and touch, then the endodermal epithelium from each side fuses ventral to the notochord. The definitive notochord is surrounded by a basal lamina which is in direct contact with the neural tube dorsally and the digestive epithelium ventrally.

The early notochordal process cells express myogenic markers transiently as they migrate beneath the epiblast but later they become epithelial, forming junctions and an outer basal lamina. The cells swell, developing an internal pressure (turgor) which confers rigidity on the notochord.

The notochord has traditionally been thought of as the axial organizer of the embryonic disc. It is suggested, from amphibian and avian studies, that progenitor cells at the node provide cell lines for **both** the notochord and the overlying medial cells of the neural plate, termed the notoplate, or (neural) floor plate (Jessell et al 1989). Although once formed the two cell populations are functionally distinct, the notochord seems to be important for the maintenance and later development of the neural floor plate, and the extension of the neural floor plate depends on the presence of underlying notochordal cells (see p. 265). Later the notochord is not so influential in neural development but it has a specific role in vertebral development providing a focus for sclerotomal migration (see p. 510).

Notochordal cells in the rhesus monkey at stage 11–12 show well-developed Golgi apparatus, mitochondria, rough endoplasmic reticulum (RER) and coated vesicles (Wilson & Hendrickx 1990), with a well-developed basal lamina. However, in the pharyngeal region of 5-week embryos the notochordal and endodermal cells are closely opposed with cell processes passing from one layer to another at the site of the bursa pharyngea. Later the basal laminae are reformed as the notochord and endoderm layers separate and mesenchymal cells become interposed (Babic

1990^[9]). The notochordal cells maintain a neural crest free zone of about 85 µm around themselves (Pettway et al 1990^[9]) in early development.

Paraxial Mesenchyme

Epiblast cells which migrate through the primitive node and rostral primitive streak during gastrulation form mesoblast cells which migrate to a position lateral to the notochord and beneath the developing neural plate. Cells ingressing through the primitive node form the medial part of this paraxial mesenchyme and cells ingressing through the rostral streak form the lateral part (3.42^[10]) (Selleck & Stern 1991^[9]). The paraxial mesenchyme extends cranially from the primitive streak to the prochordal plate immediately rostral to the notochord (3.148^[11]). Prior to somite formation it is also termed segmental plate in birds and unsegmented mesenchyme in mammals. Somitogenesis commences caudal to the otic vesicles each side of the rhombencephalon, thus somites are postotic. Paraxial mesenchyme rostral to the otic vesicle was not thought to segment; however, the appearance of somitomers has been described.

Somitomers

Experimental studies by Meier and colleagues reported in a series of papers (Meier 1979^[12], etc.) have drawn attention to the appearance of the preotic unsegmented paraxial mesenchyme when examined with scanning electron microscope (SEM). The mesenchyme shows concentric rings of cell bodies and processes forming paired, bilaminar cylinders named *somitomers* each side of the notochord and beneath the overlying neural plate. Somitomers have been identified in a wide range of vertebrates; it is suggested that they are somite precursors prior to the compaction stage (see below).


The first pair of somitomers to form lie either side of the prechordal plate with subsequent somitomers separated across the midline by the notochord. In the chick the eighth somitome, located just caudal to the otic vesicle, is the first to form a somite. All somitomers caudal to this level develop into somites.


The first seven pairs of somitomers which underlie the developing brain do not condense into somites; they remain in somitomic pattern and later the mesenchyme cells disperse to form the basal portion of the skull and all of the striated muscle of the head (3.148^[11]). There is still dispute as to the origin of the so-called preotic somites which give rise to the musculature of the eye. Christ et al (1986^[13]) suggest they derive from mesenchyme arising from the prechordal plate.



Somitogenesis


As the neural folds elevate the somitomers begin to condense forming discrete clusters of cells, the *somites*. This occurs initially at the eighth somitome which is just caudal to the midpoint of the notochordal plate. The first somite so formed is the first occipital. Somites can be seen each


side of the fusing neural tube in the human embryo from stage 9.

During somitogenesis the mesenchyme cells show changes in shape and in cell–cell adhesion becoming organized into epithelial somites. With development proceeding in a craniocaudal direction, the segmental plate is a transient and constantly changing structure, forming somites from its cranial end whilst mesenchyme, patterned into somitomeres, is being added to its caudal end by the regressing primitive streak (Packard & Meier 1983.

Experimental evidence has shown that somite induction occurs as the mesenchyme leaves the primitive streak, with cells being committed very early in development. Somites will form from cultured segmental plate with or without the presence of neural tube tissue or primitive node tissue (Packard & Jacobson 1976.


Somitogenesis (**3.131B** ) entails five main stages (after Ede & El-Gadi 1986.

- (1) The mesenchymal cells undergo *compaction*.
- (2) The outer cells of the compacted somite block form a high columnar epithelium surrounding a central cavity. Some free mesenchyme cells may remain in the cavity.
- (3) The ventral and ventromedial walls of the somite later revert to mesenchyme again, when they are termed the *sclerotome*.
- (4) The mesenchyme cells of the sclerotome migrate ventrally and medially towards the notochord, meeting the sclerotomal cells from the other side of the body. These cells begin differentiation into chondroblasts forming the vertebral bodies (see p. 267.
- (5) The remaining cells constitute the *epithelial plate of the somite*, also termed the *dermomyotome*. The dorsomedial lip of the epithelial plate folds back onto its lateral wall and the cells expand along the inside of the plate. The inner cells form myotubes and constitute the *myotome*. The dermomyotome and the definite myotome portion give rise to the hypaxial and epaxial muscles respectively.



Later cells migrating from the ventral edge of the epithelial plate of the occipital somites pass into the floor of the mouth to form the muscles of the tongue. Cells from the ventral edge of those somites opposite the developing limb buds migrate into the limbs to form the skeletal muscles of the limbs (Christ et al 1986.

Forty-four pairs of somites form caudal to the otic vesicle (postotic somites): 4 occipital, 8 cervical, 12 thoracic, 5 lumbar, 5 sacral and 8–10 coccygeal.

The somites give rise to the axial skeleton, i.e. the vertebral column and ribs from the sclerotome portion, and all the striated muscle in the body from the myotomes. Also they give rise to the muscles of the tongue, diaphragm and limbs from their ventrolateral edges (from those somites opposite the developing limbs), and the ventrolateral muscles of the trunk (from those somites between the limbs). The muscles of the head arise from the unsegmented somitomeres. Whereas


the majority of the somites produce a similar range of derivatives, the most cranial and most caudal are different. The occipital somites and the cranial somitomeres are involved in formation of the base of the skull and most of the calvaria (see p. 271 ); the caudal somites regress early.

The somites, once formed, lie lateral to the neural tube. They cause bulges in the overlying ectoderm and can be readily identified in fresh and fixed embryos. Counting somites provides a method of staging embryos; the first somites are seen at Carnegie stage 9 but by stage 13 there are more than 30 somites and accurate enumeration is difficult. At the latter stages the limb buds provide an easier external feature for staging purposes.

Somites have a specific effect on the position of the developing spinal nerves, which preferentially grow through the cranial half of the sclerotome. Even if a portion of neural tube is turned through 180°, the nerves will deviate to grow through the cranial portion of the somite (Keynes & Stern 1986 ). Note that the spinal nerves are derived from two sources, the motor nerves from the neural tube, and the sensory nerves from the neural crest. The somites affect the developing nerves still further in that addition of an extra somite causes the production of an extra dorsal root ganglion from the neural crest, and removal of a somite prevents ganglion formation. (For more details see p. 226 .)



Lateral Plate

Lateral plate is the term for the early mesoblast population lateral to the paraxial mesenchyme; it is unsegmented. These mesoblastic cells, which arise from the middle of the primitive streak (primary mesenchyme), migrate cranially, laterally and caudally to their destinations where they revert to epithelium. They form a continuous layer which adheres to the ectoderm dorsally and the endoderm ventrally and faces a new intraembryonic cavity, the intraembryonic coelom, which, becoming confluent with the extraembryonic coelom, provides a route for the circulation of coelomic fluid through the embryo. The epithelial coelomic wall thus formed becomes highly proliferative and rapidly produces a thick layer of mesenchymal cells deep to it. The mesenchymal population subjacent to the ectoderm is termed *somatopleuric mesenchyme*, and is produced by the somatopleuric coelomic epithelium. The mesenchymal population surrounding the endoderm is termed *splanchnopleuric mesenchyme*, and is produced by the splanchnopleuric coelomic epithelium.

It is important to note that these terms are only relevant caudal to the third visceral arch. Rostral to this there is a sparse mesenchymal population between the pharynx and the surface ectoderm (prior to migration of the head neural crest) with no landmarks to demarcate lateral from paraxial mesenchyme. This *unsplit lateral plate* forms the cricoid and arytenoid cartilages, the tracheal rings and the associated connective tissue (Noden 1988 .

Somatopleuric Mesenchyme

(3.51B-E )


This produces a mixed population of connective tissues and has a significant organizing effect opposite the limbs. Chick–quail chimera experiments have demonstrated that the pattern of limb development is controlled by the connective tissue cells, specifically by information contained in the somatopleuric mesenchyme (Kieny et al 1986). Regions of the limb are specified by interaction between the surface ectoderm (apical ectodermal ridge) and underlying somatopleuric mesenchyme; together these tissues form the progress zone of the limb (see p. 288). The somatopleuric mesenchyme in the limb bud further specifies the postaxial border of the developing limb. Muscles of the limbs derive from somitic precursor muscle cells (see above). The somatopleuric mesenchyme thus gives rise to the connective tissue elements of the appendicular skeleton, including the pectoral and pelvic girdles, the bones and cartilage of the limbs and their associated ligaments and tendons. Further, somatopleuric mesenchyme controls the pattern of development in the limbs, including the ectodermal specialities seen in proximal and distal parts of the limb, perhaps by mechanisms similar to the control of craniofacial development by cephalic neural crest (see below).

Splanchnopleuric Mesenchyme

(3.51B-E)

Surrounding the developing gut and respiratory tubes, it contributes connective tissue cells to the lamina propria and submucosa; also smooth muscle cells to the muscularis mucosae and muscularis externa. It has a patterning role here also as in the limbs. Recombination experiments of endodermal epithelium combined with different mesenchymal populations have shown that the splanchnopleuric mesenchyme specifies the villus type in the gut, or the branching pattern in the respiratory tract.

Intermediate Mesenchyme

This is a loose collection of mesenchyme cells found between the somites and the lateral plate (**3.51B-E **). Its development is closely related to the progress in differentiation of both the somites and the proliferating coelomic epithelium from which it derives. Intermediate mesenchyme is not present in the chick before somitogenesis and not prior to formation of the eighth somite. In embryos with eight to ten somites it is present lateral to the sixth, but does not extend cranially. The mesenchyme cells are arranged as layers, one continuous with the dorsal side of the paraxial mesenchyme and the somatopleure, the other with the ventral side of the paraxial mesenchyme and the splanchnopleure.

As development proceeds the intermediate mesenchyme forms a loosely packed cord of cells dorsolaterally which lengthens at the caudal end ultimately joining the cloaca. This is the precursor of the nephric duct. The cranial portion of this duct anlage, which gives rise to the pronephric duct in lower amniotes, degenerates whereas the middle part becomes epithelial and canalized forming the mesonephric duct.

In some amniotes the coelomic epithelium differentiates directly into specialized excretory cells, podocytes medially and a ciliated tract laterally which communicates with the nephric duct. In humans this pronephric stage is truncated and mesonephric tubules arise from proliferation and delamination of the coelomic epithelium along a strip each side of the median line. The mesonephric tubules once formed show early regional differentiation with one end forming podocytes and the other ciliated epithelium; they connect to the mesonephric duct.

The intermediate mesenchyme which forms the nephric system does not show the segmentation seen in the paraxial mesenchyme, although it has for many years been described as a segmental structure.


As already noted, the intermediate mesenchyme develops from proliferating coelomic epithelium between the splanchnopleuric and the somatopleuric coelomic epithelia. In that region the coelomic epithelium and the underlying intermediate mesenchyme together contribute to the adrenal glands and the gonads.

Angioblastic Mesenchyme

Mesoblastic cells give rise to the blood vascular and lymphatic systems of the embryo, forming the endothelial lining, the smooth muscle coat and the connective tissue adventitia (the latter may alternatively arise from splanchnopleuric mesenchyme). The vascular systems have to function precociously to fulfil the needs of the embryo as well as developing towards the vessel arrangements necessary for independent life after birth. The early endothelial channels form complex anastomotic links which may supply structures valuable to embryonic life or develop along redundant phylogenetic lines until converted to vessels appropriate for later stages of development.

The specific origin of endothelium is proving very difficult to establish in spite of the newer techniques of chimera production and immunocytochemical labelling. The rapidity with which embryos are vascularized is phenomenal and the constant modelling and remodelling of vessels compounds the difficulties of systematic study. Certainly during vascular reorganization the direction of blood flow in many vessels may reverse several times, causing obvious problems in deciding which vessels are veins and which arteries; in fact vessels may be both. It is only after the tunica media has developed that histological criteria can be used to identify the status of vessels.


Historically many theories were presented which supported the extraembryonic development of blood vessels. Blood islands appear in the yolk sac wall between the yolk sac endoderm and the extraembryonic mesoblast; these islands fuse to provide the early yolk sac circulation. Only after the formation of extraembryonic blood islands is vasculogenesis seen in the body of the embryo, giving rise to the hypothesis that all intraembryonic vessels are derived from extraembryonic, yolk sac endothelial populations that grow into the embryo. This hypothesis led to the conclusion that there are no angiogenic precursor cells within the embryo.


More recent immunological studies have demonstrated *angiogenic cells* within the early mesenchymal populations of the embryo; however they have not established the origin of these cells, i.e. whether or not they derive from yolk sac mesenchyme which has invaded the embryo. The earliest angioblastic cells are present in 1-somite chicks within the splanchnic mesenchyme around the margin of the entrance to the foregut. Later, labelled cells are present more caudally close to the endoderm of the mid- and hindgut. Later still, individual angioblasts are present in the head mesenchyme and somatopleuric mesenchyme. In a series of chimera experiments Noden (1991) has shown that ultimately all mesenchymal tissues, apart from notochord and prechordal plate, contain angioblastic cells. He notes that no ectodermal tissues, i.e. neuroepithelium and neural crest mesenchyme, contain endogenous endothelial cells. This means that crest derived mesenchyme of the face and jaw is dependent on adjacent mesenchyme for its angioblastic cells; whether this is paraxial or lateral plate mesenchyme is not clear.

Chimera experiments have further demonstrated the highly invasive nature of angioblastic cells. They are able to migrate in every direction throughout embryonic mesenchymal tissues; however, they do not enter the neural epithelium, forming instead a plexus of endothelial capillaries around the brain.

The ultimate position of endothelial vessels is believed to be patterned, like other tissues, by the mesenchymal populations of neural crest in the head, somatopleuric mesenchyme in the limbs and splanchnopleuric mesenchyme around the viscera.

Neural Crest

The neural crest is the name given to a band of epithelial cells at the outermost edges of the neural plate, between the presumptive epidermis and the neural tube; these cells are committed to a neural crest lineage before the neural plate begins folding. As a further, separate origin of mesenchyme cells in the embryo, arising after the usual formation of mesoblast from the primitive streak, the neural crest is unique. As an entity it has only a temporary existence. It develops at the time of closure of the neural tube and soon after the crest cells disperse (2.29C) , in some cases migrating over considerable distances, to a variety of different developmental fates. The extent of their diversity encompasses

- the PNS apart from the somatic motor neurons, i.e. the neurons and glia of the sensory, autonomic and enteric nervous systems
- the medulla of the adrenal gland
- all the melanocytes found in the epidermis
- nearly all of the mesenchyme of the head including the viscerocranium (see below) and page 276
- the tunica media of the aortic arch arteries.


It is interesting to note that unlike mesoblast produced from the primitive streak, none of the cells that arise from the neural crest become arranged as epithelia.

Horstadius, in a classic work published in 1950, summarized the knowledge at that time, noting that descriptions of the neural crest were very rare in textbooks. Because the cells have such a range of diverse derivatives they have been in the past very difficult to follow developmentally. Horstadius noted that it was not possible to trace the migrations and ultimate fates of the neural crest cells with the experimental methods available then. In more recent years a technique of chimera production (Le Douarin & McLaren 1984^[4]) and antibody labelling has transformed this field of embryology and given significant insights into the most fundamental developmental processes and the fates of very specific regions of the neural crest.

Neural crest cells begin as ectodermal epithelial cells at the junction of the neural plate and presumptive epidermis. The crest cell population arising from the head is larger than that found at any trunk level and gives rise to a diverse array of connective tissues in addition to peripheral neuronal, glial and pigment cells. As both the development and fate of head and trunk neural crest cells are very different they will be considered separately.

Trunk Neural Crest

This is formed as the neural tube closes, initially in the cervical region, then proceeding caudally; thus various stages of crest development can be found in the more caudal regions of the embryo. As the neural tube begins to fuse dorsally in the midline the neural crest cells lose their epithelial characteristics and junctional connections and form a band of loosely arranged mesenchyme cells immediately dorsal to the neural tube and beneath the ectoderm. Initially the crest cells mostly have their long axis perpendicular to the long axis of the neural tube; later the cell population expands laterally and around the neural tube as a sheet.


Trunk neural crest cells migrate via three routes from their position dorsal to the neural tube (3.110 ):

- ventrally
- dorsolaterally
- in a rostrocaudal direction along the aorta.


After the epithelial somites dissociate to form the sclerotome and dermomyotome, the majority of the crest cells migrate ventrally passing either into the intersomitic space or through the rostral half of the dispersing sclerotome. These crest cells do not move medially towards the notochord but continue ventrally towards the adrenal medulla, or the region of the putative sympathetic trunk or the aorta. Other crest cells move a shorter distance between the neural tube and the posterior sclerotome; it is suggested that they get trapped in this position to form the dorsal root ganglia (Bronner-Fraser 1987^[4]).


In the second migration route, crest cells pass dorsolaterally between the ectoderm and the epithelial plate of the somite into the somatopleure where they eventually form the skin melanocytes.

Head Neural Crest


These cells, unlike in the trunk, migrate before the neural tube closes. Two populations of crest cells develop: those which retain a neuronal lineage and contribute to the somatic sensory and parasympathetic ganglia in the head and neck; and those which produce extensive mesenchymal populations. Each brain region has its own crest population which migrates around the sides of the neural tube in a dorsolateral migration to reach the ventral side of the head (3.148 ). Crest cells surround the prosencephalic and optic vesicles and occupy each of the pharyngeal arches. They provide mesenchyme cells which will produce the connective tissue in the viscerocranium and parts of the neurocranium. Thus all cartilage, bone, ligament, tendon, dermal components and glandular stroma in the head derives from the head neural crest.

Head

The head is one of the most complex regions of the vertebrate body and its development is correspondingly intricate. Head development is fundamentally similar in all vertebrate groups. It involves the migration of disparate cell populations, the transient contact between cells and cell populations and contact between cells and matrix environments. The development of the vertebrate head and the mechanisms by which such development occurs are closely connected to its evolution. Indeed study of the genetic regulation of head development is providing clues to the origin of the vertebrate line (for more details on evolution of the head see p. 287 .


All vertebrates have a tripartite brain (with fore-, mid- and hindbrain) and morphological segmentation during hindbrain development. All vertebrates have essentially the same series of cranial nerves and ganglia, with the same connections to the CNS. Similarly the neurocranium of vertebrates is composed of paired sensory capsules surrounding their respective sense organs. Most significantly, all vertebrates have a significant portion of their skull and face rostral to the notochord. The developing tissue responsible for this 'prechordal' or 'new' head (Gans & Northcutt 1983 ) is the (ectodermal) neural crest. Unlike its role in the trunk, where it contributes to the sensory and autonomic nervous system, in the head the neural crest produces a significant mesenchymal population which patterns the development of the pharyngeal arches, produces the vault of the skull, induces the migration of ectodermal placodes and contributes to the sensory ganglia and sense organs in the head.

The structures that are present in the head during development appear to be segmentally organized. This segmentation may be controlled by conserved homeotic genes which have been identified in a wide range of vertebrates. However, there are still many problems with interpretation of the developmental processes operating within the head. Axial tissues and those more laterally placed seem to be induced at different times and from different precursor populations; they do not mix. Yet, other cell populations can migrate through the boundaries

between medial and lateral populations and may preferentially do so. Generally, the mechanisms of development in the head are different from those in the trunk. The extent of the difference is not as yet known. Figure 3.148  shows diagrammatic representations of the structures present in the head of a stage 11 embryo arranged in register. It should be appreciated that the head undergoes a rostrocaudal development and that therefore the structures illustrated would not be present simultaneously.



Nutrition of the Embryo




In early development the blastomeres derive their nourishment in part from stores laid down in the cytoplasm of the primary oöcyte. Such stores are possibly maintained at a high concentration but are not as extensive as those found in the yolk of most non-mammalian species. In addition it is assumed that the embryo derives nutrition from tubal and uterine secretions (Leese 1988^[1], 1989^[2]). The cleaving embryo uses pyruvate rather than glucose as an energy substrate, but switches to the utilization of glucose at the blastocyst stage (Leese & Barton 1984^[3]; Hardy et al 1989^[4]). During the preimplantation phase new protein production occurs but there is also breakdown, resulting in a slight net decrease in protein content. Similarly, lipid metabolism shows considerable changes over the preimplantation period. As well as compounds that act as metabolic precursors, the uterine tube and uterus and their respective secretions contain cytokines. Receptors for some of these have been detected in the preimplantation embryos of experimental species (DiAugustine et al 1988^[5]; Heyner et al 1989^[6]; Pampfer et al 1991^[7]; McLachlan et al 1991^[8]; Harvey & Kaye 1991^[9]; Miyazawa 1992^[10]; Wiley et al 1992^[11]; Dardik et al 1992^[12]).

Subsequently, during the process of implantation, breakdown products stemming from lysed uterine tissues may also provide a source of nutrition. Then follows a period of about two weeks during which the embryonic disc is dependent on nutrients obtained from the fluid-filled cavities of the amnion, the coelom and the yolk sac. These fluids contain products arising as a result of absorption by trophoblast from lysed uterine tissues and extravasated maternal blood. However, at an early stage in development these sources of supply are much diminished. The lumen of the neural tube is isolated by closure of the neuropores, the extraembryonic coelom becomes, relatively, greatly reduced (3.52 ) and is later shut off from the intraembryonic coelom, and the obliteration of the yolk duct separates the yolk sac from the gut. Absorption of nutrients over the surface of the embryo becomes inadequate as the surface-to-volume ratio decreases. It therefore becomes imperative that some other source should be available at an early stage. This involves the maternal circulation coming into close, although indirect, apposition with the developing embryonic circulation.

The differentiating *angioblastic* mesenchyme, in which the embryonic vessels and erythrocytes develop, is probably first formed from the deepest layer of mesenchyme which clothes the endoderm of the yolk sac early in the third week (p. 140^[13]). Slightly later, angioblastic mesenchyme can also be recognized in the connecting stalk and mesenchyme of the chorion, and it then appears also within the embryonic area. Within the angioblastic mesenchyme spaces form



and the cells lining them differentiate into typical flattened endothelial cells. Neighbouring spaces join to form capillary plexuses. Meanwhile small localized groups of mesenchymal cells project into the spaces and become cut off to form *blood islands*, their cells differentiating into embryonic erythrocytes.


The vessels formed in the chorion soon establish an intimate relationship with the maternal circulation (3.55 , p. 160 ). Vessels develop in the embryo as two longitudinal channels which, at their headward ends, invade the wall of the pericardium; the position and direction of the invasion changes with the progress of head fold formation. The two channels are the rudimentary right and left dorsal aortae and after folding their cranial ends curve ventrally in the lateral wall of the pharynx to reach the cranial end of the pericardium. Here they fuse, becoming continuous with the developing primitive tubular heart. Caudally the aortae traverse the connecting stalk as the rudimentary umbilical arteries and break up into capillaries in the chorion. The venules from the chorion converge on the stalk where they form the right and left umbilical veins, which run headwards in the somatopleure, close to the margin of the embryonic area, to reach the caudal end of the tubular heart.

The pericardial cavity never communicates directly with the extraembryonic coelom, and (before head folding) at its craniolateral limits the somatopleure and splanchnopleure are continuous (3.159A ). With formation of the head fold the mesenchymal masses extending from the surface of the pericardium are altered in disposition or even reversed and the original cranial mass comes into intimate relation with the *ventral* wall of the foregut as far as the cranial rim of the cranial intestinal portal (3.52 ). After reversal, the caudal wall of the pericardium deepens dorsoventrally; the mesenchyme between it, the gut and proximal yolk stalk forms a sheet, which is the *septum transversum*. At this stage it is bounded on its headward surface by the pericardium and on its caudodorsal surface by the foregut. On its dorsolateral surface it is limited by the bilateral pericardio-peritoneal canals, which connect the pericardium with the peritoneal cavity, and on its caudolateral surface by the single crescentic opening of the peritoneal cavity into the extraembryonic coelom. The umbilical and body wall veins, which run in the somatopleure, and the vitelline veins, which run in the splanchnopleure, meet in the junctional mesenchyme of the septum transversum and so gain the venous end of the heart. Through these various channels the early embryonic circulation is established (p. 312  et seq.). By the end of the third week the primitive cardiovascular system has been established and the heart has begun to beat so that the blood now circulates.

Fetal Membranes and Placenta

Allantois




The allanto-enteric diverticulum (3.52B,C ) arises early in the third week as a solid, endodermal outgrowth from the dorsocaudal part of the yolk sac into the mesenchyme of the connecting stalk. (For discussion of the construction of the outgrowth, see p. 151 .) It soon becomes canalized and, when the hindgut is developed, the proximal (enteric) part of the diverticulum is incorporated in its ventral wall and the distal (allantoic) part remains as the



allantoic duct and is carried ventrally to open into the ventral aspect of the cloaca or terminal part of the hindgut (3.52 ). The diverticulum, lined with endoderm, is surrounded by mesenchyme of the connecting stalk, in which the umbilical vessels develop at a slightly later stage.

In reptiles, birds and many mammals the allantoic diverticulum develops into a stalked vesicle or diffuse cord which continues expanding into the extraembryonic coelom and forms a vascular organ to which the term *allantois* should perhaps be restricted. In birds it spreads over the dorsal surface of the embryo as a flattened sac between amnion and chorion (with which it fuses) and surrounds the yolk sac. It forms the chorio-allantoic circulation, allowing gas exchange across the shell membrane and absorption of nutrients from the yolk. With the formation of the amnion the embryo is, in most mammals, separated entirely from the chorion and is not united to the chorion again until the allantoic mesenchyme spreads to become applied to its inner surface. The human embryo, however, is never wholly separated from the chorion; its caudal end is joined to the latter by a thick band of mesenchyme, the *connecting stalk*, which accordingly is regarded as precociously formed *allantoic mesenchyme*.


Amnion




The amnion is a membranous sac that surrounds the embryo; it is developed in reptiles, birds and mammals (Amniota), but not in amphibia or fishes (Anamniota).


In the human embryo the amnion appears as a cavity within the inner cell mass adjacent to the overlying trophoblast. (For details see p. 137 .) This cavity is roofed by a stratum of epithelial cells, and its floor is formed by the cells of the embryonic germ disc—continuity between the roof and floor being at the margin of the disc. The epithelial cells vary from tall columnar to flat and more squamous (Goto 1959 ). Fluid, the *liquor amnii*, occupies the amniotic cavity and increases steadily in volume as the sac gradually expands in the extraembryonic coelom (3.52 ); this continues until the coelom is obliterated, except for a small volume which is enclosed within the proximal part of the umbilical cord (the *umbilical coelom*).


Externally the amnion is covered with a thin layer of somatopleuric extraembryonic mesenchyme, which is continuous at the margins of the disc both with the splanchnopleuric extraembryonic mesenchyme covering the yolk sac and with the intraembryonic mesenchyme. Through the connecting stalk it is continuous also with the extraembryonic chorionic mesenchyme. It is commonly stated, based on morphological observations, that this extraembryonic mesenchyme is derived from the trophoblast. There is no experimental evidence for or against this in primates. In rodents, labelling studies have shown that it is derived from the embryonic ectoderm via the primitive streak (Gardner & Rossant 1979 ; Lawson & Pedersen 1992 .

Connecting Stalk and Umbilical Cord


The *connecting stalk* (3.52 ) is, as described above, a mass of precociously formed allantoic

mesenchyme, which at first connects the caudal end of the embryonic area with the chorion. Proximally (its embryonic end) it surrounds the short allanto-enteric diverticulum but it is traversed throughout its length by the umbilical (allantoic) vessels. At first its dorsal surface is covered with the amnion and its ventral surface is bounded by the extraembryonic coelom. As a result of the folding of the embryo and distension of the amnion, the embryonic end of the connecting stalk comes to lie on the ventral surface of the embryo, and its mesenchyme approaches that covering the yolk sac and its stalk. With continued expansion of the amnion, the extraembryonic coelom is largely obliterated (3.52 ) and its only remaining part surrounds the elongating yolk stalk; this part still communicates freely through the umbilicus with the intraembryonic coelom. The mesenchyme-covered surfaces of the dome and folds of the expanding amnion now reach the chorion and converge on the connecting stalk and yolk stalk (and their vessels), and the umbilical cord is formed as they meet and the two mesenchymal compartments fuse (3.52 ) thus almost completely closing off the intraembryonic coelom. A limited exocoelomic recess persists in the embryonic end of the cord (*umbilical coelom*), retaining its communication with the intraembryonic coelom, and is involved in later enteric development (see below and p. 190 )




The *umbilical cord* (3.53 ) thus consists of an outer covering of flattened amniotic epithelial cells, containing an interior mass of mesenchyme of diverse origins (see below). Embedded in the latter are two endodermal tubes—the yolk and allantoic ducts—their associated vitelline and allantoic (umbilical) blood vessels and, near its fetal end, the remains of the extraembryonic coelom mentioned above.

The *mesenchymal core* is derived from the somatopleuric extraembryonic mesenchyme covering the amniotic folds; the splanchnopleuric extraembryonic mesenchyme of the yolk stalk which carries the vitelline vessels and clothes the endodermal yolk duct; and similar allantoic mesenchyme of the connecting stalk which clothes the allantoic duct and carries initially two umbilical arteries and two umbilical veins. These various mesenchymal compartments fuse and are gradually transformed into the loose connective tissue (*Wharton's jelly*) which characterizes the more mature cord. The tissue consists of widely spaced elongated fibroblasts separated by an intercellular space filled with a copious matrix consisting of a delicate three-dimensional meshwork of fine collagen fibres surrounded by a dilute ground substance (Parry 1970 )

. The latter contains a variety of hydrated glycosaminoglycans and is particularly rich in hyaluronic acid. In specimens which have been excised before fixation and staining, the fibroblasts present stellate profiles.


The part of the extraembryonic coelom (the *umbilical coelom*) included in the base of the umbilical cord acts as a sac which receives the normal *umbilical hernia* of the midgut, developing in the embryo between the sixth and tenth weeks (p. 190 )

. After the disappearance of this hernia the extraembryonic coelomic sac is normally obliterated.


The yolk sac becomes located between the amnion and chorion as they fuse near the placental attachment of the cord (3.52 , 54 , 63 )

. It continues to grow slowly and is sometimes found at term in this site, as a small vesicle usually less than 5 mm in diameter. The yolk stalk



and its contained endodermal duct and accompanying vessels gradually elongate with growth in length of the umbilical cord. The duct and vessels slowly degenerate and they have usually disappeared by midpregnancy.

The endodermal allantoic duct, which is confined to the proximal end of the growing cord, also elongates and thins but may persist as an interrupted series of epithelial strands until term. At the umbilicus the proximal strand is often continuous with the median intra-abdominal *urachus*, which in turn continues into the apex of the bladder (p. 201.

Usually, the embryonic right umbilical vein disappears in the early months of pregnancy (exceptionally only one artery may persist). The vessels of the umbilical cord are rarely straight but usually show a twisted conformation which may exist as either a right- or left-handed cylindrical helix. The number of turns involved may be relatively few or, at the other extreme, may even exceed 300. Their causation has been variously ascribed to unequal growth of the vessels, or to torsional forces imposed by fetal movements; their functional significance is obscure; perhaps their pulsations and contractions (see below) assist the venous return to the fetus in the umbilical vein. When fully developed the umbilical vessels, particularly the arteries, are provided with a strong muscular coat which contracts readily in response to mechanical stimuli. The outermost bundles pursue an interlacing spiral course so that, when they contract, they produce shortening of the vessel and thickening of the media, with folding of the interna and considerable narrowing of the lumen. This action may account for the periodic sharp constrictions of contour—the so-called *valves of Hoboken*—which often characterize these vessels.

When fully developed, the umbilical cord is on average some 50 cm long and 1–2 cm in diameter, but the length is subject to great variation (20–120 cm). Exceptionally short or long cords are associated with fetal problems and complications during labour as discussed by Benirschke and Kaufmann (1990). The cord usually attaches to the placenta but in a minority of cases velamentous insertion is observed (i.e. into the membranes) and may be associated with vulnerability to injury and attendant obstetric complications. This is discussed in detail by the same authors.

Implantation

As noted (p. 132), fertilization occurs in the lateral or ampullary part of the fallopian tube, and is followed around 26–40 hours later by the first cleavage. The dividing preimplantation embryo is conveyed along the tube to the uterine cavity by ciliary action of the tube aided by muscular tubal contractions; the journey occupies about 3 days. After entering the uterine lumen the morula develops an internal cavity and becomes a blastocyst, still surrounded by the zona pellucida. By this stage two distinctive groups of cells have emerged: one constitutes the *inner cell mass* which will form the embryo and contribute to the extraembryonic membranes; the second is the *trophectoderm*, flattened polyhedral cells surrounding the blastocyst cavity which have ultrastructural features typical of a transporting epithelium (Enders & Schlafke 1965). The cells covering the inner cell mass are known as *polar trophectoderm* and those surrounding the

blastocyst cavity as *mural trophectoderm*. From these cells the trophoblast of the mature placenta is derived.

It has been suggested that the mesenchyme of the chorion is derived either from the trophoblast or the extraembryonic endoderm. Experimental evidence addressing this question is naturally lacking in the human but the mesoblastic layer of the murine chorion are derived from the embryonic ectoderm via the primitive streak (Rossant & Croy 1985; Rossant 1986). This is in agreement with the morphological observations of Luckett (1978) for the human.

Escape of the blastocyst from the zona is a prerequisite for its implantation in the uterine mucosa (see p. 136). This may involve the production of a trypsin-like enzyme and the presence of local weaknesses in the zona (Perona & Wasserman 1986; Lindenberg & Hyttel 1989). In the interval between ovulation and blastocyst arrival in the uterine cavity, preimplantation changes also occur in the uterine mucosa. These progestational changes are detailed later (p. 162).

The process of implantation involves an initial *attachment* of the polar trophectoderm to the endometrial luminal epithelium. Following this it *penetrates* the epithelium and underlying basal lamina and implants into the stroma, using a combination of motile and locally degradative activities.

A major problem in establishing the mechanism of the initial interaction is that in the earliest *in situ* implantation sites available for examination, implantation is already underway (Hertig et al 1956; Pijnenborg 1990). Early implantation in primates is initiated by a close approach of the trophoblastic plasma membrane to the tips of the microvilli and irregular surface protrusions of the uterine epithelial cells. The microvilli shorten and disappear and, for a period, there is a close mutual apposition between the contours of the trophoblast and the uterine epithelial cell surface. From *in vitro* studies initial implantation in the human seems to involve attachment of trophectoderm to the endometrial epithelium followed by intrusion between the epithelial cells without their destruction (Lindenberg et al 1986, 1990). The syncytium sends finger-like projections between adjacent epithelial cells towards the underlying basal lamina, the two layers becoming closely interlocked by the formation of numerous tight junctions between them. Some authors have suggested that fusion between these two cell types may occur (Denker 1990).

The subepithelial basal lamina is penetrated by the trophoblast. There is evidence for production by preimplantation embryos of proteases that degrade basal lamina extracellular matrix molecules (Glass et al 1983) including type IV collagen (Behrendtsen et al 1992). In rodents it appears that the underlying stroma contributes to the local breakdown of the epithelial basal lamina and matrix (Blankenship & Given 1992). Implantation of the human blastocyst continues with erosion of maternal vascular endothelium and glandular epithelium and phagocytosis of secretory products until the blastocyst occupies an uneven *implantation cavity* in the stroma (*interstitial implantation*). It is not known precisely when syncytiotrophoblast first appears, but by the time contact is established with the stroma, the outermost cells are syncytial (Hertig et al 1956). In the early postimplantation phase, the maternal surface is resealed by a combination of re-epithelialization and formation of a plug that has been suggested to consist of

fibrin.

Trophoblast and Chorion


If fertilization and implantation are successfully accomplished, a hormone secreted by the syncytiotrophoblast, *human chorionic gonadotrophin* (hCG), prolongs the life of the corpus luteum, which continues to secrete progesterone and oestrogens during approximately the first 2 months of pregnancy. Thereafter these and other hormonal functions are the province of the definitive placenta (Devroey et al 1990^[4]). Menstruation does not occur and the endometrium, now known as the *decidua of pregnancy*, thickens further to form a suitable nidus for the conceptus. During the first few days after implantation, hCG appears in the urine where its presence is used as the basis for tests for early pregnancy.

The syncytiotrophoblast increases rapidly in thickness over the embryonic pole with a progressively thinner layer over the rest of the wall towards the abembryonic pole. As the blastocyst implants, the syncytiotrophoblast invades and digests the uterine tissues, including glands and the walls of maternal blood vessels (see 3.36^[5], 38^[6] and Böving 1959^[7], 1963^[8]). Microvillus-lined clefts and lacunar spaces develop in the syncytiotrophoblastic envelope (days 9–11 of pregnancy) and establish communications with one another. Early, many of them contain maternal blood (3.36^[5], 38^[6]) derived from dilated uterine capillaries and veins, the walls of which have been partially destroyed. As the conceptus grows, the lacunar spaces enlarge, becoming confluent to form an initial *intervillous space*; their microvillous trophoblastic walls are converted at first into an irregular spongework or *labyrinth* (known inappropriately as primary villi; days 12–13). This is then invaded first with cytotrophoblast and then with mesenchyme (days 13–15) to form a radial array of *secondary placental villi*. Villous strands extend from the syncytial layer of the chorion across the intervillous space. On their embryonic aspect is a layer of cytotrophoblast, lined by vascularized fetal mesenchyme. The villous strands extend to the layer of peripheral trophoblast which is apposed directly to the excavated maternal tissues. Through spaces in the latter, extravasated maternal blood continues to enter the intervillous space. However, there is evidence that the maternal vascular circuit that connects the uterine arterial supply via the intervillous space to maternal veins is not fully functional until late in the first trimester (Hustin & Schaaps 1987^[9]).

As the intrasyncytial lacunae are developing, a *column* of proliferating cytotrophoblast extends from the *chorionic plate* and breaks through the syncytium to make direct contact with the maternal stroma (before day 15). Further cytotrophoblast proliferation then occurs laterally so that neighbouring outgrowths meet to form a spherical *cytotrophoblastic shell* around the conceptus (3.35^[10], 38^[6], 55^[11]). Capillaries form within the mesenchymal core (3.55^[12]) and establish connections with the radicles of the umbilical vessels in the general mesenchyme of the chorion. The heart now beats, establishing circulation between the yolk sac, the embryo and the chorio-allantoic placenta (days 18–22). Each villus now consists, from its base in the chorionic plate and throughout much of its extent, of a vascularized mesenchymal core, covered by a single (*Langhans*) layer of cytotrophoblast, which is again ensheathed by a layer of syncytium. These *tertiary* (or *mesenchymal*) villi proceed into a sequence of developmental changes continuing to term (see below). Near the maternal interface they contain no

mesenchymal core but comprise a solid *cytotrophoblastic cell column*, continuous peripherally with the trophoblastic shell. At its periphery, the developing placenta thus consists of tertiary chorionic villi connected to the maternal stroma by cytotrophoblast columns—so-called *anchoring villi*.

Continuing growth of the cytotrophoblast columns occurs and single mononuclear cells detach from their distal tips and infiltrate the maternal decidua (Pijnenborg et al 1980^[1], 1981^[2], 1990^[3]). This process occurs in two phases: an initial infiltration of the basal decidua, with *interstitial extravillous trophoblast* tending to be more populous in the vicinity of maternal spiral arteries; and a second wave of migration by which the extravillous trophoblast reaches the inner one-third of the myometrium. At the same time, cytotrophoblast from the shell penetrates into and migrates along the inner walls of maternal spiral arteries (*endovascular extravillous trophoblast*), again penetrating by the 18th week as deep as the inner myometrial segments. The interstitially migrating cells appear to have the capacity to invade arteries from their periphery. The function (possibly the sole function) of this infiltrative behaviour by cytotrophoblast appears to be in the remodelling of the maternal arteries with loss of smooth muscle and associated elastic and collagenous matrix and its replacement with non-resistive fibrinoid, thus allowing for an expansion of the vessels and as much as a 20-fold increase in the flow of blood into the intervillous space. Common pregnancy pathologies including intrauterine growth retardation, pre-eclampsia and spontaneous abortion are all associated with incomplete vascular remodelling probably arising from a failure of penetration by extravillous trophoblast (Khong 1991^[4]).

Expansion of the whole conceptus is accompanied by *radial growth* of the villi and, simultaneously, an integrated *tangential growth* with expansion of the trophoblastic shell and increased complexity and branching of the villous tree continuing to term. Eventually each stem forms a complex consisting of a single *trunk* (*truncus*) attached by its base to the chorion, from which arise distally, second and third order branches (intermediate and terminal villi; Castellucci et al 1990^[5]). *Terminal villi* are specialized for exchange between the fetal and maternal circulations (3.56 ). Each terminal villus commences as a syncytial outgrowth which, as it continues to grow, is invaded by cytotrophoblastic cells which then develop a core of fetal mesenchyme; this is finally vascularized by fetal capillaries (i.e. each villus passes through primary, secondary and tertiary grades of histological differentiation). Thus the germinal *cytotrophoblast*, by multiplicative growth, can continue to add additional cells which fuse with the overlying syncytium and allow the expansion of the haemochorial interface. The terminal villi continue to form and branch, within the confines of the definitive placenta (see below) throughout gestation, projecting in all directions into the intervillous space.

As these changes proceed, the intervillous space, at first spanned by the early villous stems and their branches, is increasingly permeated by growing free villi. It contains the circulating maternal blood, and is bounded:

- On its *fetal aspect* by a chorionic plate consisting of syncytial, cytotrophoblastic and mesenchymal layers of the chorion, the latter carrying radicles of the umbilical vessels and fusing laterally with the mesenchyme of the expanding amnion.
- On its maternal aspect by a *basal plate* consisting of *incomplete peripheral syncytium*

with an outer *cytotrophoblastic shell* and *columns* extending deeper into the maternal decidual stroma. The trophoblast and adjacent decidua are enmeshed in layers of *fibrinoid* and basement membrane-like extracellular matrix to form a complex junctional zone (Enders 1968; Aplin 1991b; Damsky et al 1992). Where a discrete layer of fibrinoid is present between the trophoblastic shell and decidual stroma, this is known as *Nitabuch's layer*.

- Crossing it from chorionic to basal plates, the main trunks of the *villous stems* dividing into their intermediate and terminal villi; the trunk and its branches may be regarded as the essential structural, functional and growth unit of the developing placenta.

From the third week until about the second month of pregnancy the entire chorion is covered with villous stems which are thus continuous peripherally with the trophoblastic shell which is in close apposition with **both** the decidua capsularis and the decidua basalis. The villi adjacent to the basal decidua, however, are stouter, longer and show a greater profusion of terminal villi. As the conceptus continues to expand, the decidua capsularis is progressively compressed and thinned, the circulation through it is gradually reduced and, accordingly, the adjacent villi slowly atrophy and disappear. This process starts at the abembryonic pole and by the end of the third month the abembryonic hemisphere of the conceptus is largely denuded. Eventually the whole chorion apposed to the capsularis is smooth (the *chorion laeve*). In contrast, the villous stems of the disc-shaped region of chorion apposed to the decidua basalis increase greatly in size and complexity (the *chorion frondosum*), and together with the basalis constitute the *definitive placental site*.

Further consideration of the placenta must now be deferred until the preparation of the uterine tissues for the implantation and development of the blastocyst has been briefly described.

Cyclical Changes in the Uterus


Throughout the period of reproductive life (i.e. from about the fifteenth to the forty-fifth year), except during pregnancy and lactation, a series of closely interrelated cyclical changes occur in the ovary, uterus and vagina. Each cycle extends over a period of about 28 days. In the ovarian cycle, which is described more fully elsewhere (pp. 122, 1865), one follicle usually reaches full maturity, ruptures and releases its secondary oöcyte during this period. The wall of the follicle is then transformed into an important endocrine gland, the *corpus luteum* (p. 1865). About 10 days after ovulation the corpus luteum begins to regress, then ceases to function and is replaced by fibrous tissue.




The changes of the *uterine cycle* (*menstrual cycle*) chiefly involve the lining endometrium of the body and fundus of the uterus and may, for convenience, be divided into three phases:



- menstrual

- proliferative
- secretory (Noyes et al 1950^[1]; Wynn 1977^[2]; Cornillie et al 1985^[3]; Dockery et al 1988a^[4], 1990^[5]; Aplin 1989^[6]; Buckley & Fox 1989^[7]).

The secretory phase coincides with the luteal phase of the ovarian cycle.

In the *menstrual (haemorrhagic) phase* the superficial part of the endometrium, next to the free surface, is shed piecemeal, leaving mainly the basal zone, adjacent to the uterine muscle (3.57 ). Approximately two-thirds to three-quarters of the thickness of the endometrium may be shed. Outwardly this phase is marked by a discharge of blood with necrotic epithelial and stromal debris from the uterus through the vagina. This discharge, the menstrual flow, lasts some 3–6 days.

In the early *proliferative phase*, and even before the menstrual flow ceases, the epithelium from the persisting basal parts of the uterine glands grows luminally over the denuded surface of the endometrium. Re-epithelialization is complete by days 5–6 after the start of menstruation. Initially the tissue is only 1–2 mm thick and lined by low cuboidal epithelium. The glands are straight and narrow with short columnar cells. The apical cell surface contains microvilli, and some ciliated cells are present. The stroma is dense and contains small numbers of lymphocytes amongst the larger population of mesenchymally-derived cells (3.57 ). During the 10–12 days of the proliferative phase there is a growth of the endometrium associated with the presence in the bloodstream of oestrogen. This is produced by the ovary (3.58 ) and acts through receptors present on both the stromal and epithelial cells of the endometrium. Mitoses are present and the glands become distinctly tortuous. Their lining epithelium becomes tall columnar (3.57 ).

Ovulation occurs about 14 days before the onset of the next menstrual flow. The changes occurring in the secretory phase depend upon the presence in the bloodstream of *progesterone* and *oestrogens*, secreted by the corpus luteum (3.58 ). Steroid receptors in the endometrium respond by activating a programme of new gene expression to produce, in the following 7 days, a highly regulated sequence of differentiative events presumably required to prepare the tissue for implantation (Dockery et al 1988a^[4],b^[5]; Smith et al 1989^[8]; Aplin 1989^[6], 1991a^[9]; Bell 1990^[10]). Part of the response is direct, but there is evidence that some of the effects may be mediated through growth factors (Nelson et al 1991^[11]; Tabibzadeh 1991^[12]). The first morphological effects of progesterone are evident 24–36 hours after ovulation. In the early secretory phase glycogen masses (known incorrectly as 'subnuclear vacuoles') appear in the basal cytoplasm of the epithelial cells lining the glands, where they are often associated with lipid. Nuclei are thus displaced towards the centre of the cells (3.59 ). Giant mitochondria appear and are associated with semi-rough endoplasmic reticulum. A prominent nuclear channel system is present. A notable increase in polarization of the gland cells occurs with Golgi apparatus and secretory vesicles accumulating in the supranuclear cytoplasm. Nascent secretory products may be detected immunohistochemically within the gland cells.

By the *midsecretory phase* the endometrium may be up to 6 mm deep. The basal epithelial glycogen mass is progressively transferred to the apical cytoplasm, allowing the return of nuclei to the cell base. The Golgi apparatus becomes dilated and products including glycogen, mucin and other glycoproteins are released from the glandular epithelium into the lumen by a combination of apocrine and exocrine mechanisms, reaching a maximum approximately 6 days after ovulation (Smith et al 1989^[4]). These secretory changes are considerably less pronounced in the basal gland cells and the luminal epithelium than in the glandular cell population of the functionalis. In the late secretory phase glandular secretory activity declines. Production by gland cells of certain specific secretory products however is not observed until the mid- and late-secretory phases (Bell 1990^[5]).

Progestational effects on the stroma are also evident (Wienke et al 1968^[6]; Cornillie et al 1985^[7]; Aplin et al 1988^[8]; Dockery et al 1990^[9]). In the early secretory phase nuclear enlargement occurs and the packing density of the resident mesenchymal cells increases due in part to the increase in volume of gland lumens and onset of secretory activity in the epithelial compartment. In the midsecretory phase, a notable oedema appears with a corresponding decrease in the density of collagen fibrils. At the same time the endoplasmic reticulum and Golgi apparatus become more prominent, and there is evidence for new synthesis of collagen as well as its endocytosis and degradation.

In the late secretory phase decidual differentiation occurs in the superficial stromal cells surrounding blood vessels. This transformation includes rounding of the nucleus and an increase in the cytoplasmic volume with a concurrent increase and dilatation of the rough endoplasmic reticulum (RER) and Golgi systems and cytoplasmic accumulation of lipid droplets and glycogen. The cells begin to produce basal lamina components including laminin and type IV collagen. Features of the fully differentiated decidual cell are described in the next section.

Three strata can now be clearly recognized in the endometrium (3.57 


- *stratum compactum*, next to the free surface, in which the necks of the gland are but slightly expanded and the stromal cells show a distinct decidual reaction
- *stratum spongiosum*, where the uterine glands are tortuous, dilated and ultimately only separated from one another by a small amount of interglandular tissue
- a thin *stratum basale*, next to the uterine muscle, containing the tips of the uterine glands embedded in an unaltered stroma.


Towards the end of this period, as regression of the corpus luteum occurs, those parts of the stroma showing a decidual reaction and the glandular epithelium both undergo degenerative changes and the endometrium often diminishes in thickness. These degenerative changes precede the phase of bleeding.


During *menstruation*, blood escapes from the superficial vessels of the endometrium forming small haematomata beneath the surface epithelium which raise it. Blood and necrotic

endometrium then begin to appear in the uterine lumen. The shedding of the endometrium starts at the surface and extends into the deeper layers. The amount of tissue lost is variable, but usually the stratum compactum and most of the spongiosum are desquamated.

The endometrium is regenerated from the stratum basale and that part of the spongy layer which remains, the surface epithelium being reformed with remarkable rapidity.

During the proliferative, early and midsecretory phases of the cycle, the bone-marrow derived cells present in endometrium are mainly macrophages and classic T cells, with very few B cells. In the late secretory phase, an unusual, large, granular lymphocyte population is recruited to the tissue (Starkey et al 1991 ) and is found mainly in the stromal compartment.

The *vascular bed* of the endometrium undergoes significant changes during the menstrual cycle. The arteries to the endometrium arise from a *myometrial plexus* and consist of short *straight* vessels to the basal portion of the endometrium and more muscular *spiral* arteries to its superficial two-thirds. The capillary bed consists of an endothelium with a basal lamina that is discontinuous in the proliferative phase, becoming more distinct by midsecretory phase (Roberts et al 1992 ). Pericytes are present, some of which resemble smooth muscle cells, and these are sometimes enclosed within the basal lamina. The pericytes make contact with the endothelial cells by means of cytoplasmic extensions that project through the basal lamina. Enlargement of the pericytes is evident beginning in the early secretory phase and leading to a conspicuous cuff of cells in the mid- and late-secretory phases. The venous drainage, consisting of narrow perpendicular vessels which anastomose by cross branches, is common to both the superficial and basal layers of the endometrium. The arterial supply to the basal part of the endometrium remains unchanged during the menstrual cycle. The spiral arteries to the superficial strata, however, lengthen disproportionately, become increasingly coiled and their tips approach more closely the uterine epithelium during the secretory phase of the menstrual cycle. This leads to a slowing of the circulation in the superficial strata with some vasodilation. Immediately before the menstrual flow these vessels begin to constrict intermittently causing stasis of the blood and anaemia of the superficial strata. During the periods of relaxation of the vessels, blood escapes from the devitalized capillaries and veins, thus causing the *menstrual haemorrhage*.

If fertilization of the ovum does not occur, the corpus luteum undergoes degeneration. The breakdown of the endometrium follows this cessation of function and is due to the reducing levels of progesterone and oestrogen (p. 1873 )

Decidua

If fertilization occurs, chorionic gonadotrophin secreted by the conceptus rescues the corpus luteum and progesterone levels continue to rise, preventing menstruation and stimulating decidual differentiation of the endometrial stroma. During decidualization the interglandular tissue increases in quantity; it contains a substantial population of leucocytes (large granular lymphocytes, macrophages and T cells) distributed amongst large decidual cells. The latter are mesenchymally-derived stromal cells which have accumulated varying amounts of glycogen,

lipid and vimentin-type intermediate filaments in their distended cytoplasm. They may contain one, two or sometimes three nuclei. The light microscopic appearance of these cells is rounded, but although they lack the long thin cytoplasmic projections typical of fibroblasts, the cell shape varies depending on the local packing density, which is widely variable. Frequently, rows of club-like cytoplasmic protrusions enclosing granules are found at the periphery. Outside the cell is a characteristic capsular basal lamina (Wynn 1974; Aplin 1989; Enders 1991).

Decidualization of the endometrial stroma occurs in humans regardless of the presence of a conceptus, as indicated by:

- the similar changes seen in the presence of an ectopic pregnancy
- the pseudodecidual changes observed in the late secretory phase of a non-conception cycle
- the decidualization observed after prolonged treatment with progesterone
- the ability of cultured stromal cells to decidualize in vitro in the presence of progesterone.

It is interesting to note that in rats, decidualization of stromal cells requires not only steroidal sensitization, but also an initiation signal from the embryo. Rats do not menstruate, but rather exhibit an oestrous cycle. The rather advanced differentiation achieved by the human stroma in the absence of a pregnancy may conceivably be related to the need for the seemingly wasteful sloughing of large parts of the tissue at menstruation.

Decidual cells produce a range of secretory products (Bell 1986; Fazleabas et al 1991; Seppala et al 1992) including insulin-like growth factor, binding protein 1 and prolactin, which diffuse across the chorio-amnion and may be detected in amniotic fluid in the first trimester of pregnancy. They may also be internalized by trophoblast. These secretions probably play a role in the maintenance and growth of the conceptus in the early prehaemochorial phases of postimplantational development, when placentation is transiently *deciduochorial*. Decidualization also involves extensive remodelling of the stromal extracellular matrix. In addition to production of the capsular basal lamina, degradation and new synthesis of fibrillar collagen occur with a net decrease in the fibril density. Extracellular matrix, growth factors and protease inhibitors produced by the decidua probably modulate the degradative activity of the trophoblast (Lala & Graham 1990; Librach et al 1991; Bischof et al 1992; Seppala et al 1992) and support placental morphogenesis and access of the placenta to the maternal blood supply. Thus formation of the haemochorial placenta requires a developmental progression specified in the trophoblast but dependent on the maternal environment for its correct expression. It also depends on the lack of immunological rejection of the semi-allogeneic conceptus, which is achieved by the absence of polymorphic histocompatibility antigens (HLA) on trophoblast but may also depend in some part on the specialized immune cell populations present in the decidua and their cytokine network (Starkey et al 1991).

Decidual differentiation is not evident in the stroma at the earliest stages of implantation, and it

may not be until a week later that fully differentiated cells are present (Enders 1991^[1]). Distinctive names are now applied to different regions of the decidua: the part covering the conceptus is the *decidua capsularis*, the part between the conceptus and the uterine muscular wall is the *decidua basalis*, and it is here that the placenta is subsequently developed; the part which lines the remainder of the body of the uterus is the *decidua parietalis* (3.61^[2]). However, there is no evidence that their respective resident maternal cell populations exhibit different properties in these various locations.

Coincidentally with the growth of the embryo and the expansion of the amnion (p. 142^[3]), the decidua capsularis is thinned and distended (3.60^[4], 61^[5]) and the space between it and the decidua parietalis gradually obliterated. By the second month of pregnancy the three endometrial strata recognizable in the premenstrual phase, compactum, spongiosum and basale, are better differentiated and easily distinguished. In the spongiosum the glands are compressed and appear as oblique slit-like fissures lined by low cuboidal cells. By the beginning of the third month of pregnancy the capsularis and parietalis are in contact: by the fifth month the capsularis is greatly thinned, while during the succeeding months (3.62^[6]) it virtually disappears.


Definition of the Human Placenta

The human placenta is initially *labyrinthine* as the early villous stems are formed, but becomes secondarily *villous* with the development of generations of terminal villi. Maternal blood bathes the surfaces of the chorion which bound the intervillous space and it is thus defined as *haemochorial*, distinguishing it from the different grades of fusion between the maternal and fetal tissues which exist in many other mammals (*epitheliochorial*, *syndesmochorial*, *endotheliochorial* and others; Mossman 1987^[7]).

The chorion is vascularized by the allantoic blood vessels of the body stalk and the human placenta is termed *chorio-allantoic* (whereas in some mammals a *choriovitelline* placenta either exists alone or supplements the chorio-allantoic variety). Finally, the human placenta is said to be *deciduate* because maternal tissue is shed with the placenta and membranes at term as part of the afterbirth (see below).



An exhaustive account of the growth, dimensional changes, vasculature and haemodynamics, cell varieties, ultrastructure and histochemistry of the placenta, and the physiological aspects of placental transfer and its status as a metabolic store and endocrine gland, lies beyond the scope of the present volume. What follows is necessarily an abbreviated account of selected topics and the interested reader should consult the profusion of original papers devoted to these subjects. The classic work of Boyd and Hamilton (1970^[8]) and the more recent volume of Benirschke and Kaufmann (1990^[9]) provide unrivalled sources of information and extensive bibliography. A survey of placental transfer mechanisms is to be found in Sibley and Boyd (1992^[10]).

The Placenta at Term

The expelled placenta (3.63 ) is a flattened discoidal mass with an approximately circular or oval outline, with an average volume of some 500 ml (range 200–950 ml), average weight about 500 g (range 200–800 g), average diameter 185 mm (range 150–200 mm), average thickness 23 mm (range 10–40 mm) and an average surface area of about 30 000 mm². Thickest at its centre (the original embryonic pole) it rapidly diminishes in thickness towards its periphery where it continues as the chorion laeve.

Macroscopically, its *fetal* or *inner surface*, covered by amnion, is smooth, shiny and transparent and the mottled appearance of the subjacent chorion, to which it is closely applied, can be seen through it. The umbilical cord is usually attached near the centre of the fetal surface, and branches of the umbilical vessels radiate out under the amnion from this point, the veins being deeper and larger than the arteries. Beneath the amnion and close to the attachment of the cord, the remains of the yolk sac can sometimes be identified as a minute vesicle, up to 5 mm in diameter, with a fine thread—a vestige of the yolk stalk—attached to it.

The *maternal surface* is finely granular and mapped into some 15–30 lobes by a series of fissures or grooves. The lobes are often somewhat loosely termed *cotyledons* (but see also below) and the grooves correspond to the bases of incomplete *placental septa* which become increasingly prominent from the third month onwards. They extend from the maternal aspect of the intervillous space (the basal plate) towards, but not quite reaching, the chorionic plate. The septa are complex structures comprising components of the cytotrophoblastic shell and residual syncytium along with maternally derived material including decidual cells, occasional blood vessels and gland remnants, collagenous and fibrinoid extracellular matrix and, in the later months of pregnancy, foci of degeneration. The nature of the maternal surface of the expelled placenta is of course determined by the tissue plane of separation of the placenta at parturition.

Studies of the human placenta include morphometric analysis, surface architecture using scanning electron microscopy, ultrastructural studies of angioarchitecture and possible mechanisms whereby the maternal placental circulation is controlled. The ultrastructure of biopsies taken from placental uterine beds which were presumed to be normal has been reviewed (Wynn 1974 ) . A detailed investigation into the connective tissue 'skeleton' of the placenta, with a helpful bibliography, has been provided by Ockleford and Wakely (1982 ) .

Separation of the Placenta

After delivery of the fetus the placenta becomes separated from the uterine wall and, together with the so-called 'membranes', is expelled as the *afterbirth*. Separation takes place along the plane of the stratum spongiosum and extends beyond the placental area, detaching:

- the villous placenta with associated fibrinoid matrix and small amounts of decidua basale
- the chorio-amnion together with a superficial layer of the decidua capsularis/parietalis.

The chorio-amnion is continuous with the placenta at its margin and constitutes the '*membranes*' familiar in obstetrics. The process of separation requires rupture of many uterine vessels but their torn ends are closed by the firm contraction of the muscular wall of the uterus after delivery of the placenta and membranes and thus, under normal circumstances, postpartum haemorrhage is limited in amount. When the placenta and membranes have been expelled, a thin layer of stratum spongiosum is left as a lining for the uterus, but it soon undergoes degeneration and is cast off in the early part of the puerperium. A new epithelial lining for the uterus is then regenerated from the remaining stratum basale.

Chorio-Amnion

Between the tenth and twelfth weeks of pregnancy the amniotic cavity expands and the chorion frondosum regresses to form the chorion laeve which is in turn apposed to the decidua capsularis. During the same period the amnion and chorion fuse to form the chorio-amnion, and this avascular membrane persists to term.

The inner surface of the amnion consists of a simple cuboidal epithelium with a microvillous apical surface beneath which is a cortical web of intermediate filaments and microfilaments. There are no tight junctional complexes between adjacent cells and cationic dyes penetrate between the cells as far as the basal lamina (King 1982^[1]). The intercellular clefts present scattered desmosomes, but elsewhere the clefts widen and contain interlacing microvilli. These features are consistent with selective permeability properties. The epithelium synthesizes and deposits extracellular matrix into the compact layer of acellular stroma located beneath the basal lamina, as well as the basal lamina itself (Lister 1968^[2]; Wynn & French 1968^[3]; Aplin et al 1985^[4], 1986^[5]; Campbell et al 1990^[6]). The compact layer varies considerably in thickness between different specimens. Beneath the compact layer is a fibroblast layer and then a spongy layer that abuts the chorion laeve. It is likely that lateral movement of the amnion occurs relative to the chorion in response to mechanical stress, and that this is effectively lubricated by the spongy layer.

The chorion at term consists of an inner cellular layer containing fibroblasts and a reticular layer of fibroblasts and Hofbauer cells that resembles the mesenchyme of an intermediate villus. The outer layer consists of cytotrophoblast 3–10 cells deep resting on a pseudobasal lamina that extends beneath and between the cells (Bourne 1963^[7]; Aplin & Campbell 1985^[8]). Obliterated villi are occasionally seen within the trophoblast layer and represent the remnants of villi present in the chorion frondosum of first trimester. Although the interface between the trophoblast and decidua parietalis is uneven, no trophoblast infiltration of the parietalis occurs.

The *liquor amnii* increases in quantity up to the sixth or seventh month and then diminishes slightly; at the end of pregnancy it is usually somewhat less than a litre. It provides a buoyant medium which supports the delicate tissues of the young embryo and allows free movement of the fetus during the later stages of pregnancy. It also diminishes the risk to the fetus of injury from without. It contains less than 2% of solids, including urea, inorganic salts, a small amount of protein and frequently a trace of sugar. The liquor is derived from multiple sources: secretions

from amnion epithelium; filtration of fluid from maternal vessels via the parietal decidua and amniochorion; filtration from the fetal vessels via the chorionic plate or the umbilical cord; fetal urine. In early pregnancy, diffusion from intracorporeal vessels via fetal skin provides another source (Benirschke & Kaufmann 1990^[1]). In the early stages it resembles blood plasma in composition and is probably formed largely by transport across the amniotic membrane but as pregnancy advances it becomes progressively more dilute, partly by the addition of fetal urine. Glycoprotein secretions from amniotic epithelium include fibronectin. It has been shown experimentally that there is a considerable and rapid flux of water across the amniotic membrane. There is rapid exchange between the amniotic fluid and maternal and fetal circulations, probably via the placenta and fetal kidneys. By the end of the third month the expanding amnion has extensive contact with the chorion laeve and only these thin membranes separate the amniotic fluid from the decidua parietalis, the tissues and vessels of which provide another route for the exchange of water and dissolved substances (Plentl 1958^[2]; Bell 1986^[3]).

Secretory products of maternal decidua (see below), including prolactin and insulin-like growth factor binding protein (IGF-BP1), are present in the liquor (Seppala et al 1992^[4]). A volume of amniotic fluid in excess of 2 litres is generally considered to be abnormal and constitutes *hydramnios*. A deficiency is termed *oligamnios*. Both conditions may be associated with fetal abnormalities; for example, fetuses with agenesis of the kidneys or atresia of the lower urinary tract are often associated with oligamnios.

Fetal swallowing of amniotic fluid is a normal occurrence; the fluid is absorbed into the fetal circulation and passes the placental barrier into the maternal circulation. Cases of oesophageal atresia or anencephaly, in which swallowing is impossible or impaired, and open spina bifida are often associated with hydramnios. With these neural defects, impaired swallowing is accompanied by direct discharge of cerebrospinal fluid (CSF) into the amniotic liquor. In fetuses with spina bifida and some other neural tube defects the concentration of alpha-fetoprotein in the amniotic fluid is exceptionally high and is used to diagnose these abnormalities (Brock 1976^[5]). Fluid is also produced in the fetal lungs; however, most of this fluid remains in the lungs as a mechanical effect of the amniotic fluid pressure. Pulmonary hypoplasia at birth may be caused by severe congenital urinary obstruction (see p. 181^[6]).

Placental Tissues

These are arranged as a chorionic plate, a basal plate and, between the two, the villous stems, their branches and the intervillous space (3.55^[7], 56^[8], 57^[9], 58^[10], 59^[11], 60^[12], 61^[13], 62^[14], 63^[15], 64^[16]).

Chorionic Plate


This is covered on its fetal aspect by the amniotic epithelium, on the stromal side of which is a connective tissue layer carrying the main branches of the umbilical vessels. Adjacent to this is a diminishing layer of cytotrophoblast and finally the inner syncytial wall of the intervillous space.

The connective tissue layer derives from fusion between mesenchyme-covered surfaces of amnion and chorion and is more fibrous and less cellular than Wharton's jelly of the umbilical cord, except near the larger vessels. The latter radiate and branch from the cord attachment (with variations in the branching pattern), until they reach the bases of the trunks of the villous stems, which the branches enter and then arborize within the intermediate and terminal villi. There is no anastomosis between vascular trees of adjacent stems but, in contrast, the two umbilical arteries are normally joined by some form of substantial transverse (Hyrtil's) anastomosis at, or just before they enter, the chorionic plate.

Basal Plate

From the fetal to maternal aspect this consists of:

- the outer wall of the intervillous space comprising in different places syncytium, cytotrophoblast or fibrinoid matrix
- Rohr's stria of fibrinoid
- what remains of the cytotrophoblastic shell
- Nitabuch's stria of fibrinoid
- maternal decidua.

Nitabuch's stria and the basal decidua contain cytotrophoblast and multinucleate trophoblast giant cells originating from the mononuclear cytotrophoblast population that infiltrates the basal decidua during the first 18 weeks of pregnancy. These cells penetrate as far as the inner one-third of the myometrium, but can often be observed at or near the decidual–myometrial junction. They are not found in the parietal decidua nor the adjacent myometrium. Thus the placental-bed giant cell appears to be a differentiative end stage in the extravillous trophoblast lineage (Aplin 1991b .

The striae of fibrinoid are irregularly interconnected and variable in prominence. Strands pass from Nitabuch's stria into the adjacent decidua. The latter contains basal remnants of the endometrial glands, large and small decidual cells scattered in a connective tissue framework which also supports an extensive venous plexus.

Throughout the second half of pregnancy the basal plate is thinned and progressively modified, with a relative diminution of the decidual elements, and increasing deposition of fibrinoid and admixture of fetal and maternal derivatives.

Intervillous Space

Through the various layers of the basal plate the maternal blood vessels approach and reach the intervillous space. The spiral arteries of the endometrium open through gaps in the cytotrophoblastic shell and peripheral syncytium. However, they probably do not open directly

into the intervillous space until as late as the tenth week. At term, from the inner myometrium to the intervillous space, the walls of most spiral arteries consist of fibrinoid matrix within which cytotrophoblast is embedded. This allows expansion of the arterial diameter to give an increased flow of blood which is privileged in being independent of vasoconstrictors. Endothelial cells, where present, are often hypertrophic.

The veins which drain the blood away from the intervillous space pierce the basal plate and join tributaries of the uterine veins. The presence of a marginal venous sinus, which has hitherto been described as a constant feature, occupying the peripheral margin of the placenta and communicating freely with the intervillous space, has not been confirmed.

In the macaque monkey, radio-opaque material injected into the aorta passes in spurts or jets to the intervillous space and at sufficient pressure to drive it towards the chorion, thus preventing a short circuit of arterial blood into the venous openings. The openings of the coiled arteries show intermittent activity. Myometrial contractions alter the pressure in the intervillous space and promote placental venous drainage (Ramsey et al 1963; Martin 1965).

Placental Lobes and Lobules

The placental *lobes* are demarcated by the grooves on its maternal surface, and they correspond in large measure to the major branches of distribution of umbilical vessels, particularly well seen in specimens X-rayed after intravascular injection of radio-opaque media. However, the application of the term cotyledon to these major lobes does not correspond directly to its usage in comparative placentology, where cotyledon refers to scattered discontinuous patches of villous chorion interspersed with non-villous chorion (as found for example in cows).

The fetal cotyledon of the human placenta evidently corresponds to a major villous stem and its branches. Early in pregnancy, the chorion bears some 800–1000 of such stems but as pregnancy advances, with the formation of the chorion laeve and possibly some fusion between adjacent stems, the number is progressively reduced until only about 60 persist in the placental area in the last months of pregnancy.

Structure of a Villus

Chorionic villi are the essential structures involved in exchanges between mother and fetus and, accordingly, the villous tissues separating fetal and maternal blood are of crucial functional importance. From the chorionic plate, progressive branching occurs into the villous tree, stem villi giving way to intermediate and terminal villi. For a complete description of the development and structure of the villous tree, readers are referred to Benirschke and Kaufmann (1990).

Each villus has a core of connective tissue containing collagen types I, III, V and VI as well as fibronectin. Cross-banded fibres (30–35 nm) of type I collagen are often found as bundles. Type III collagen is present as thinner (10–15 nm) beaded fibres forming a meshwork that often encases the larger fibres. Collagens V and VI are present as 6–10 nm fibres closely associated

with collagens I and III. The basal lamina-associated molecules laminin and collagen type IV are present in the stroma in association with fetal vessels, as well as in the trophoblast basal lamina (Amenta et al 1986^[1]). Overlying this matrix are ensheathing cyto- and syncytial trophoblast bathed by the maternal blood in the intervillous space (3.55^[2], 64^[3], 65^[4], 66^[5]). Cohesion between the cells of the cytotrophoblast and also between this layer and the syncytium is provided by numerous desmosomes between their apposed plasma membranes.

In earlier stages, the cytotrophoblast forms an almost continuous layer on the basal lamina, but after the fourth month it gradually expands itself producing syncytium (Midgley et al 1963^[6]). As the cytotrophoblast decreases, the syncytium becomes directly adjacent to the basal lamina over an increasingly large area and becomes progressively thinner. A few cytotrophoblastic cells, usually disposed singly, persist until term. In the first and second trimester cytotrophoblastic sprouts are present, covered in syncytium, and represent a stage in the development of new villi. At the tips of anchoring villi cytotrophoblast columns extend from the villous basal lamina to the maternal decidual stroma as described previously.

The cells of the *villous cytotrophoblast* (*Langerhans cells*) are pale-staining with only a slight basophilia. Ultrastructurally, they show a rather electron-translucent cytoplasm, with relatively few organelles: clusters of ribosomes, narrow cisternae of rough endoplasmic reticulum, a number of large mitochondria, variable Golgi apparatus and intermediate filaments particularly in association with the desmosomes. Between the desmosomes, the membranes of adjacent cells are separated by about 20 nm. Sometimes the intercellular gap widens to accommodate microvillous projections from the cell surfaces; the gap occasionally contains patches of fibrinoid.

A smaller population of intermediate cytotrophoblast may also be found in the chorionic villi. This postmitotic population represents a state of partial differentiation between the cytotrophoblast stem cell and the overlying syncytium (Jones & Fox 1991^[7]).

The syncytial cytoplasm is more strongly basophilic and possesses many ultrastructural features which distinguish it from the cytoplasm of the Langerhans cells. Where the plasma membrane adjoins basal lamina it is often complexly infolded into the cytoplasm, whereas the surface bordering the intervillous space is set with numerous long microvilli, the cores of which show linear densities. These microvilli are responsible for the brush border of light microscopy.

The syncytial cytoplasm is exceedingly complex and more electron-dense than that of Langerhans cells. It contains a wealth of free ribosomes, cisternae of granular endoplasmic reticulum, scattered representations of the Golgi complex, mitochondria, a cytoskeleton of microfilaments and a profusion of vesicles and vacuoles, some smooth and some coated, of a wide size range, numerous lysosomes, phagosomes and other electron-dense inclusions (Jones & Fox 1991^[7]). It is an intensely active tissue layer across which most transplacental transport must occur. It is also responsible for the secretion of a range of placental proteins into the maternal circulation. These include chorionic gonadotrophin, chorionic somatomammotropin (formerly known as placental lactogen) and others.

Glycogen is held to be present in both layers of the trophoblast at all stages but it is not always possible to demonstrate it by histochemical means. Lipid droplets are also present in both layers and free in the core of the villus. In the trophoblast they are found principally within the cytoplasm but also occur extracellularly between cytotrophoblast and syncytium, or between the individual cells of the cytotrophoblast and also in the basal lamina. These droplets diminish in number with advancing age and may represent fat in transit from mother to fetus, or a pool of precursors for steroid synthesis. Membrane-bound granular bodies of moderate electron-density also occur in the cytoplasm, particularly in the syncytium. Some of these are probably secretion granules. The lysosomes and phagosomes are evidently concerned with the degradation of materials engulfed from the intervillous space.

On the free surface of the villus various types of specialization occur, though care must be taken to distinguish these from artifacts caused by tangential sectioning (Cantle et al 1987^[4]). In the immature placenta, syncytial sprouts are found representing the first stages of development of new terminal villi. These later become invaded by cytotrophoblast and villous mesenchyme. Occasionally, adjacent syncytial sprouts make contact and fuse to form slender syncytial bridges. Syncytial sprouts are also present in the term placenta, but here the enclosed nuclei are largely degenerative. Syncytial knots represent similar aggregates of degenerative nuclei, but here not associated with a projection from the villous surface. This may represent a sequestration phenomenon involving removal of senescent nuclear material from adjacent metabolically active areas of syncytium. The sprouts may become detached, forming *maternal syncytial emboli* which pass to the lungs. It has been computed that there is a passage of some 100 000 of such sprouts daily into the maternal circulation. In the lungs they provoke little local reaction and apparently disappear by lysis but they may, on occasion, form foci for neoplastic growth.

Fibrinoid deposits are frequently found on the villous surface in areas lacking syncytiotrophoblast; this appears to be a repair mechanism in which the fibrinoid forms a wound surface that is subsequently re-epithelialized by trophoblast (Nelson et al 1990^[4]). The extracellular matrix glycoprotein tenascin is localized in the stroma adjacent to these sites (Castellucci et al 1991^[4]).

The core of the villus contains small and large reticulum cells, fibroblasts, and large phagocytic *Hofbauer cells* which are more numerous in early pregnancy (Jones & Fox 1991^[4]). Early mesenchymal cells probably differentiate into small reticulum cells which in turn produce fibroblasts or large reticulum cells. The small reticulum cells appear to delimit a collagen-free stromal channel system through which Hofbauer cells migrate (Martinoli et al 1984^[4]). Mesenchymal collagen increases from a network of fine fibres in early mesenchymal villi to the densely fibrous stroma of stem villi of the second and third trimester. After about the 14th week, the stromal channels found in immature intermediate villi are infilled by collagen to give the fibrous stroma characteristic of the stem villus.

The fetal vessels include arterioles and capillaries. Their endothelial cells contain fine cytoplasmic filaments and they extend bulbous projections into the lumen. Pericytes may be found in close association with the capillary endothelium. From late first trimester the vessels are

surrounded externally by a periendothelial basal lamina membrane. From the second trimester, and later in terminal villi, dilated thin-walled capillaries are found immediately adjacent to the villous trophoblast, the two basal laminae having apparently fused to produce a vasculo-syncytial interface.

Maturation and Functions of the Placenta

In the early stages of placental development the blood in the fetal vessels is separated from the maternal blood in the intervillous space by the fetal vascular endothelial cells, the connective tissue of the villus, the subepithelial basal lamina and its covering of cyto- and syncytial trophoblast. These constitute a *placental barrier* interposed between the bloodstreams, but it is a selectively permeable barrier and allows water, oxygen and other nutritive substances and hormones to pass from mother to fetus, and some of the products of excretion to pass from fetus to mother.

Throughout pregnancy, the placenta increases its surface area and thickness, with accompanying increases in the size, length and complexity of branching of the villous stems (Benirschke & Kaufmann 1990). At term, the placental diameter varies between 200–220 mm, the mean placental weight is 470 g, its mean thickness is 25 mm and the total villous surface area exceeds 10 m². The placental barrier becomes reduced in thickness during gestation. After the fourth month the villous syncytium comes into direct apposition to the subepithelial basal lamina over an increasing area (80% at term) and it also becomes thinner. The fetal capillaries approach the surface of the terminal villi and become dilated.

The mechanism of transfer of substances across the placental barrier is complex. The volume of maternal blood circulating through the intervillous space has been assessed at 500 ml per minute (Assali et al 1960). Simple diffusion suffices to explain gaseous exchange. Transfer of ions and other water soluble solutes is by paracellular diffusion, transcellular diffusion and transport, although the relative importance of each of these for most individual solutes is unknown, and the paracellular pathway is morphologically undefined. Glucose transfer involves facilitated diffusion and active transport mechanisms carry calcium and at least some amino acids. The fat-soluble and water-soluble vitamins are likely to pass the placental barrier with different degrees of facility, and indeed it is known that the water-soluble vitamins B and C pass readily. Water is interchanged between fetus and mother (in both directions) at about 3.5 litres per hour. The transfer of substances of high molecular weight such as complex sugars, some lipids, hormonal and non-hormonal proteins varies greatly in rate and degree, and is not so readily understood. Energy-dependent selective transport mechanisms including receptor-mediated transcytosis are probably involved.

Lipids may be transported unchanged through and between the cells of the trophoblast to the core of the villus. The passage of maternal antibodies (immunoglobulins) across the placental barrier confers some degree of passive immunity on the fetus. In this instance it is widely accepted that transfer is by micropinocytosis. Investigation of transplacental mechanisms is complicated by the fact that the trophoblast itself is the site of synthesis and storage of certain

substances, e.g. glycogen. For comprehensive reviews of placental transfer mechanisms consult Sibley and Boyd (1992^[1]) and Faber and Thornburg (1983^[2]).

The placenta is an important endocrine organ; some steroid hormones, various oestrogens, β endorphins, progesterone, hCG and human chorionic somatomammotropin (hCS)—also known as placental lactogen (hPL)—are synthesized and secreted by the syncytium. The trophoblast is rich in birefringent lipids and cytochemical methods show that it also contains enzyme systems which are associated with the synthesis of steroid hormones.

It has been suggested that leucocytes may migrate from the maternal blood through the placental barrier into the fetal capillaries. It has also been shown that some fetal and maternal red blood cells may cross the barrier (Dancis 1959^[3]). The former may have important consequences, for example in rhesus incompatibility (p. 1407^[4]).

The majority of drugs are small molecules that are sufficiently lipophilic to pass the barrier. Many are apparently tolerated by the fetus, but some may exert grave teratogenic effects on the developing embryo (e.g. thalidomide). A well-documented association exists between maternal alcohol ingestion and fetal abnormalities (Sadler 1990^[5]). Addiction of the fetus can occur to substances of maternal abuse such as cocaine and heroin.

Finally, a wide variety of bacteria, spirochaetes, protozoa and viruses (including human immunodeficiency virus, HIV) are known to pass the placental barrier from mother to fetus, although the mechanism of transfer is uncertain. The presence of maternal rubella in the early months of pregnancy is of especial importance in relation to the production of congenital anomalies (see p. 333^[6]).

Placental Variations

As a rule the placenta is attached to the posterior wall of the uterus near the fundus, with its centre in or near the median plane. The site of attachment is determined by the point where the blastocyst becomes embedded but the factors on which this depends are not yet understood. The placenta may be attached at any point on the uterine wall, offering no complications to a normal labour unless it is so low down that it overlies the internal os uteri, in which case serious antepartum haemorrhage may occur, especially if it is nearly central in position. This occurs in about 0.5% of pregnancies and is known as *placenta praevia*. (*Extrauterine* sites of implantation are discussed on p. 136^[7].)


The umbilical cord, although usually attached near the centre of the organ, may reach it at any point between its centre and margin, the latter known as a *battledore* placenta. Occasionally the cord fails to reach the placenta itself and ends in the membranes in its vicinity. With such a *velamentous insertion* of the cord, the larger branches of the umbilical vessels traverse the membranes before they reach and ramify on the placenta. A small *accessory* or *succenturiate* placental lobe is occasionally present, connected to the main organ by membranes and blood vessels; it may be retained in utero after delivery of the main placental mass and prolong

postpartum haemorrhage. Occasionally other degrees of division occur (*bipartite* or *tripartite* placentae). Other variations include *placenta membranacea*, in which villous stems and their branches persist over the whole chorion, and *placenta circumvallata*, in which its margin is undercut by a deep groove. Pathological forms of adherence or penetration include *placenta accreta*, with exceptional adherence to the decidua basalis, *placenta increta*, in which the myometrium is invaded, and *placenta percreta*, when the invasion by placental tissue has passed completely through the uterine wall.

At birth, when ligation of the umbilical cord is delayed, the blood volume of the child is, on the average, appreciably greater than it is when the ligation is applied at the earliest possible moment (Yao et al 1969^[4]). It appears that in the former case much of the blood in the fetal placental vessels is transferred from the placenta to the fetus.

Development of Individual Systems

Embryonic development has so far been considered as a whole, but, as the definition of its structures proceeds, overall description becomes so complicated as to actually impede the clarity of appreciation of the events occurring. It is, hence, customary and convenient to limit attention to individual systems in their further development; but it must never be overlooked that the analysis of a whole organism into such divisions, however attractive on morphological and functional grounds, is largely a product of the sequential nature of human perception. Not only do the several systems into which we divide the organism develop simultaneously; they also interact and modify each other. This necessary interdependence is not only supported by the evidence of experimental embryology but is also emphatically demonstrated by the phenomena of growth anomalies, which cut across the artificial boundaries of systems in most instances. For these reasons it is most desirable that the development of any one individual system should be frequently related to others, especially those most closely associated with it (both spatiotemporally and causally).

So far the development of the embryo has been taken to an age of between 3 and 4 weeks, the stage of early somite formation, equivalent to Horizons X or XI on the scale established by the studies of Streeter and others (1942^[4], 1945^[4], 1948^[4], 1949^[4]) and of stages 10–11 of O'Rahilly and Muller (1987^[4]). As it is notoriously difficult to age embryos with total accuracy, stages of development have been generally used in this section. A particular stage is not an alternative way of indicating the developmental age of an embryo: a stage conveys the developmental status of many of the systems in concert. No one criterion could place a particular embryo within one stage or another: the stage is estimated from examination of a number of key structures throughout the body. 3.31  shows a graph of developmental stage and time.

The commencement of the development of the following systems begins at the body plan, pharyngula, stage (see p. 100^[4]). It is only partly constricted from the yolk sac, but the head and tail folds are well formed, with enclosure of the foregut and hindgut (proenteron and metenteron). The forebrain projection dominates the cranial end of the embryo; the

buccopharyngeal membrane and cardiac prominence are caudal and ventral to this. The intraembryonic mesenchyme has begun to differentiate with the paraxial mesenchyme undergoing segmentation into somites. Neural groove closure is progressing to a neural tube and is separated from the dorsal aspect of the gut by the notochord. The earliest blood vessels have appeared and a primitive tubular heart occupies the pericardium. The chorionic circulation is soon to be established, after which the embryo rapidly becomes completely dependent for its requirements upon the maternal bloodstream. The intraembryonic part of the coelom consists of the transmedian pericardial cavity, leading dorsocaudally into right and left pericardioperitoneal canals (coelomic ducts). The canals occupy mesenchyme dorsal to the septum transversum, caudal to which they expand into the peritoneal cavity. This establishes, for a time, a free communication with the extraembryonic coelom (3.49, 50). The development of all of the systems described in the remainder of this section begins at this stage.

Respiratory and Gastrointestinal Systems

Primitive Gut

The primitive gut is divided, by head and tail folding, into three main compartments; the *foregut* extends from the buccopharyngeal membrane (a localized region where ectoderm and endoderm are in direct opposition see p. 146) to its opening, or continuation, into the central midgut region via the cranial intestinal portal (3.67). Foregut derivatives are: part of the buccal cavity, the pharynx (and numerous subregions), the respiratory system, the oesophagus, stomach, superior and proximal half of the descending part of the duodenum, the liver, gallbladder and biliary duct systems, the pancreas and ducts. The site of the original cranial intestinal portal is immediately caudal to the common hepatopancreatic ampulla and papilla. The *midgut* extends between the intestinal portals being, in the early embryo, in wide communication with the yolk sac. With constriction of the connection to the yolk sac the midgut becomes tubular and lengthens being destined to form the remaining duodenum, jejunum, ileum, caecum and appendix, ascending colon and much of the transverse colon. The *hindgut* extends from the region of the putative splenic flexure to (and a little beyond) the cloacal membrane (a caudal region where the ectoderm and endoderm are in direct opposition, see p. 146). Its gut derivatives are the descending colon, sigmoid colon, rectum and anal canal to the level of the anal valves. The caudoventral part of the hindgut is continuous with the allantois; it separates from the alimentary hindgut and contributes to the urinary bladder, urethra and associated glands.

At a cellular level the primitive embryonic gut is formed from three sources:

- (1) The *splanchnopleuric coelomic epithelium*. Initially this is a proliferative epithelium which forms the splanchnopleuric mesenchymal populations; later it forms the outer, serosal or visceral epithelial layer.
- (2) The *endodermal epithelium* forms the epithelium of the mucosa, the lining cells of

adjoining ducts and the secretory cells of the associated glands.

- (3) The intervening *splanchnopleuric mesenchyme* forms all of the structures and tissue between the epithelial layers, i.e. the outer connective tissue layers, the muscularis externa and submucosal connective tissue; the muscularis mucosae and lamina propria of the mucosa; and the local angiogenic tissue which gives rise to the blood and lymphatic vessels.

The splanchnopleuric mesenchyme provides by far the greatest cell mass to the primitive gut. Generally the splanchnopleuric mesenchyme patterns the development of the endodermal epithelium both spatially and temporally; it contributes to the external shape of the primitive gut structures and modifies the shape of the splanchnopleuric coelomic epithelium, and therefore of the coelom. (For the derivations of these tissues see p. 145.) The gut is innervated by migrating *neural crest* cells which form the *enteric plexuses*. Lymphoid tissue becomes incorporated into the embryonic gut, both by assimilation of individual cells within the epithelium of the mucosa, and also by colonization of the submucosa by germinal centres of lymphoid tissue.

Primitive Foregut

Buccal Cavity


This derives from both ectodermal and endodermal regions. The rostral growth of the embryo and formation of the head fold causes the pericardial area and buccopharyngeal membrane to come to lie on the ventral surface of the embryo (p. 148). With further expansion of the forebrain dorsally, and bulging of the pericardium ventrally, together with enlargement of the facial prominences laterally, the buccopharyngeal membrane becomes depressed forming the base of a hollow, the *stomodeum* or *primitive buccal cavity* (3.68). At the end of the fourth week (stage 12) the membrane breaks down and a communication is established between the stomodeum and cranial end of the foregut (future oropharynx). No vestige of the membrane is evident in the adult, and this embryonic communication should not be confused with the permanent oropharyngeal isthmus. The epithelium of the lips and gums, salivary glands and the enamel of the teeth are ectodermal in origin, from the stomodeal walls, but the epithelium of part of the tongue and adnexa, developed in the posterior floor of mouth and pharynx, is derived from endoderm. The development of the teeth and gums is described on page 1712.

The pharyngeal arches grow in a ventral direction and lie progressively between the stomodeum and pericardium; with the completion of the mandibular prominences and the development of the maxillary prominences (p. 277), the opening of the stomodeum assumes a pentagonal form, bounded cranially by the frontonasal prominence, caudally by the mandibular prominences and laterally by the maxillary prominences (3.68). With the inward growth and fusion of the palatine processes (3.146), the stomodeum is divided into a nasal and a buccal part. Along the free margins of the prominences bounding the mouth cavity appears a shallow groove, and the ectoderm in its floor thickens and invades the underlying mesenchyme; it divides into a

medial *dental lamina* and a lateral *vestibular lamina*. The central cells of the latter degenerate and the furrow becomes deepened. It is now termed the *labio gingival groove* or *sulcus*; its inner wall contributes to the formation of the alveolar processes of the maxillae and the mandible and their gingivae, while its outer wall forms the lips and cheeks.

Structures in the wall of the oral cavity, i.e. *mucous glands*, *salivary glands*, *teeth* and *taste buds*, are formed by ectoderm/mesenchymal interactions. Similarly the buccal epithelium is so formed but results in a non-keratinized layer in contrast to the outer ectodermal layer which forms the keratinized layer of skin.


Teeth

These are formed by an ectoderm/mesenchyme interaction and are dealt with, more appropriately, under development of the viscerocranium (see p. 274 ).


Salivary Glands

These arise from the epithelial lining of the mouth. The *parotid gland* can be recognized in human embryos 8+ mm long (stage 15) as an elongated furrow running dorsally from the angle of the mouth between the mandibular and maxillary prominences. The groove, which is converted into a tube, loses its connection with the epithelium of the mouth, except at its ventral end, and grows dorsally into the substance of the cheek. The tube persists as the *parotid duct* and its blind end proliferates in the local mesenchyme to form the gland. Subsequently the size of the oral fissure is reduced by partial fusion between the maxillary and mandibular prominences and the duct opens thereafter on the inside of the cheek at some distance from the angle of the mouth. The *submandibular gland* is identifiable in human embryos 13 mm long as an epithelial outgrowth from the floor of the *linguogingival groove* (see below) into the mesenchyme. It increases rapidly in size, giving off numerous branching processes which later acquire lumina. At first the connection of the submandibular outgrowth with the floor of the mouth lies at the side of the tongue, but the edges of the groove in which it opens come together, from behind forwards, and form the tubular part of the *submandibular duct*. As a result, the orifice of the duct is shifted forwards till it is below the tip of the tongue, close to the median plane. The *sublingual gland* arises in embryos about 20 mm long as a number of small epithelial thickenings in the linguogingival groove and on the groove's lateral side, which later closes to form the submandibular duct. Each thickening canalizes separately; many of the multiple sublingual ducts open separately on the summit of the sublingual fold, others join the submandibular duct.

Tongue

This appears as a small median elevation, named the *median tongue bud* (*tuberculum impar*), in the floor of the pharynx before the pharyngeal arches meet ventrally; it subsequently becomes incorporated in the anterior part of the tongue. A little later two oval *distal tongue buds* (*lingual swellings*) appear on the inner aspect of the mandibular prominences. They meet each other in front, and caudally they converge on the median tongue bud, with which they fuse (3.69 ). A




sulcus forms along the ventral and lateral margins of this elevation and deepens, internal to the future alveolar process of the mandible, to form the *linguogingival groove*, while the elevation constitutes the anterior or buccal (presulcal) part of the tongue. Caudal to the median tongue bud, a second median elevation, the *hypobranchial eminence* (copula of His), forms in the floor of the pharynx, and the ventral ends of the fourth, the third and, later, the second pharyngeal arches converge into it. A transverse groove separates its caudal part to form the epiglottis, while ventrally it approaches the presulcal tongue rudiment, spreading in the form of a V, and forming the posterior or pharyngeal part of the tongue. In the process the third arch elements grow over and bury the elements of the second arch, excluding it from the tongue. As a result the mucous membrane of the pharyngeal part of the tongue receives its sensory supply from the glossopharyngeal, the nerve of the third arch. In the adult the union of the anterior and posterior parts of the tongue approximately corresponds to the angulated *sulcus terminalis*, its apex at the *foramen caecum*, a blind depression produced at the time of fusion of the constituent parts of the tongue, but also marking the site of ingrowth of the median rudiment of the thyroid gland.

At first the tongue consists of a mass of mesenchyme covered on its surface by ectoderm and endoderm. During the second month occipital myotomes migrate from the lateral aspects of the myelencephalon and invade the tongue to form its musculature. They pass ventrally round the pharynx to reach its floor accompanied by their nerve (the hypoglossal) (see p. 1256 )

The composite character of the tongue is indicated by its innervation. Impulses from and to the anterior, buccal part are mediated by: (1) the lingual nerve, derived from the post-trematic nerve of the first arch (mandibular nerve) and (2) the chorda tympani, often held to be the pretrematic nerve to the first arch. The posterior, pharyngeal part of the tongue is innervated by the glossopharyngeal, the nerve of the third arch and its root, near the epiglottis, by the vagus.

The sulcus terminalis cannot be distinguished earlier than the 52-mm stage according to some observers. The vallate papillae appear at about the same time, increasing in number until the 170-mm stage. Serial reconstructions also suggest that the territory of the glossopharyngeal nerve extends considerably beyond these papillae.

Thyroid Gland

This gland is first identifiable in embryos of about 20 somites, as a median thickening of endoderm in the floor of the pharynx between the first and second pharyngeal pouches and immediately dorsal to the aortic sac (Davis 1923 ). This area is later invaginated to form a median diverticulum which appears late in the fourth week in the furrow immediately caudal to the median tongue bud (3.69 ). It grows caudally as a tubular duct the tip of which bifurcates and subsequently the whole mass divides into a series of double cellular plates, from which the isthmus and the lateral lobes of the thyroid gland are developed. The *primary thyroid follicles* differentiate by reorganization and proliferation of the cells of these plates. *Secondary follicles* subsequently arise by budding and subdivision (Norris 1916 ). These primary and secondary endodermal cells are the progenitors of the follicular parenchyma proper. The claim that the fourth pharyngeal pouches contribute thyroid tissue to the lateral lobes of the gland was long

disputed and perhaps seemed unlikely on the grounds of comparative embryology. (But see below—the *ultimobranchial body* and the derivation of *parafollicular* or *C cells*.)

The original diverticulum, its bifurcation and generations of follicles invade the hypobranchial neural crest mesenchyme. From the latter are derived the thin connective tissue capsule, thinner 'interlobular' septa and delicate perifollicular investments. These carry the main vascular, characteristic fenestrated capillaries, lymphatics and autonomic nerve supply.

The connection of the median diverticulum with the pharynx is termed the *thyroglossal duct*. The site of its initial continuation with the endodermal floor of the mouth is marked by the foramen caecum. From here it extends caudally in the median line ventral to the primordium of the hyoid bone, behind which it later forms a recurrent loop. The distal part of the duct commonly differentiates variably as the pyramidal lobe and levator muscle (or 'suspensory' fibrous band) of the thyroid. The remainder fragments and disappears, but the lingual part is often identifiable until late in fetal life and may branch and give rise to miniature salivary glands (Boyd 1964). Occasionally parts of the midline thyroglossal duct persist (occurring in lingual, suprahyoid, retrohyoid, or infrahyoid positions). They may form aberrant masses of thyroid tissue, cysts, fistulae or sinuses, usually in the midline (see p. 1892). A *lingual thyroid* situated at the junction of the buccal and pharyngeal parts of the tongue is not uncommon, but nodules of glandular tissue may also be found other than in the midline, e.g. laterally placed posterior to sternocleidomastoid, and, on occasion, below the level of the thyroid isthmus (see p. 1892).


Pharyngeal Pouches

The development of the pharyngeal arches during stages 10 to 13 (see p. 187) causes morphological changes in the primitive rostral foregut resulting in a widened orifice at the putative mouth, rapidly narrowing caudally. The foregut rostrally is compressed dorsoventrally such that there is limited, or virtually no, true lateral wall. Between the individual arches, in the early stages, the ectoderm and endoderm are transiently closely apposed with little intervening mesenchyme. Externally such regions are termed *pharyngeal* (or *branchial*) *clefts*; internally they are referred to as *pharyngeal pouches*.

The close proximity of the ectoderm and endoderm is maintained between the first cleft and pouch which becomes the *tympanic membrane*, with minimal mesenchyme between the layers. The *first pouch* and, some maintain, part of the *second pouch*, i.e. its dorsal part, together expand as the *tubotympanic recess* which gives rise to the middle ear system (see below). The relationship between subsequent clefts and pouches diverges with mesenchyme intervening; the endoderm of the pouches thickens and evaginates into localized regions of neural crest and unsplit lateral plate mesenchyme.

The *second pouch* is much reduced in dimensions compared to the first and its ventral part is the focus of lymphoid development as the *palatine tonsil*. A generalized ring of lymphoid tissue develops in the primitive foregut at this region, resulting in the median pharyngeal *tonsil*


(adenoid), the bilateral *tubal tonsils* and the *lingual tonsil* on the posterior part of the tongue.

The *third pouch* gives rise to the *thymus* ventrally and the *parathyroid III* dorsally, whereas the *fourth pouch* produces the *parathyroid IV* and an *ultimobranchial body*. The complex of the dorsal and ventral portions of the fourth pouch plus the lower ultimobranchial body is termed the *caudal pharyngeal complex* (3.70 ).

Tonsils

Derived from the ventral parts of the second pharyngeal pouches which lie between the tongue and the soft palate; the endoderm lining these pouches grows into the surrounding mesenchyme as a number of solid buds. These buds are excavated by degeneration and shedding of their central cells, and thus the tonsillar *fossulae* and *crypts* are formed. Lymphoid cells accumulate around the crypts and become grouped as lymphoid follicles. A slit-like *intratonsillar cleft* extends into the upper part of the tonsil and is possibly a remnant of the second pharyngeal pouch.

Thymus

Derived from the endoderm of the ventral part of the third pharyngeal pouch on each side (3.70 ), the thymus cannot be recognized prior to the differentiation of the inferior parathyroid glands (see below), which occurs when the embryo is 10–12 mm long (stage 16), but thereafter it is represented by two elongated diverticula which soon become solid cellular masses and grow caudally into the surrounding neural crest mesenchyme. Ventral to the aortic sac the two thymic rudiments meet and are subsequently united by connective tissue only; the rudiments themselves remain unfused. The connection with the third pouch is soon lost, but the stalk may persist for some time as a solid, cellular cord.

The development of thymic tissue from the ventral recess of the fourth pharyngeal pouch probably occurs in a proportion of embryos, although this has been denied by some authorities (Weller 1933^[1]; Norris 1938^[2]). Thymic tissue developing from this site is usually found near but outside the thyroid gland in close association with the superior parathyroid gland. An ectodermal contribution to the thymus, probably of placodal origin, occurs in some mammals but a similar contribution in man is conjectural (Garrett 1948^[3]).

Vascularized mesenchyme, including lymphoid stem cells, invades the cellular mass of the endodermal thymus and becomes partially lobulated. The cells of the cytotreticulum and the concentric corpuscles of the thymus are endodermal in origin. The epithelial character of these cells is more obvious in fetal life; some are even ciliated (Sebuwufu 1968^[4]). Lymphoid cells enter and colonize the thymus from the haemopoietic tissue stem cells during the third month.

At birth the thymus is large relative to total body weight. Its absolute weight increases in the first 2 years after birth, but its relative weight decreases. There is little change thereafter until about the seventh year, when rapid growth again occurs to reach a maximum at about 11 years. After

this it begins to decline to an adult weight which is very variable but averages 12–15 g. In old age the gland shrinks still further, especially after wasting diseases. For this and other reasons it is rarely identifiable in the preserved cadaver of the aged (Keynes 1954^[4]; Lasi 1959^[5]; and p. 1429^[6]).

Parathyroid Glands



These are also derivatives of the endoderm and adjacent mesenchyme. Prior to the appearance of the thymic rudiment from the third pharyngeal pouch, the epithelium on the dorsal aspect of the pouch and in the region of its duct-like connection with the cavity of the pharynx becomes differentiated as the primordium of the *inferior parathyroid gland*, recognizable by its cells, which stain more lightly than the other endodermal cells lining the pouch. Although the connection between the pouch and the pharynx is soon lost, the connection between the thymic and parathyroid rudiments persists for some time, and the latter passes caudally with the developing thymus. The *superior parathyroid glands* develop in a similar manner from the dorsal recess of the fourth pharyngeal pouches. They come into relation with, and appear anchored by, the lateral lobes of the thyroid gland and thus remain cranial to the parathyroid glands derived from the third pouch. The mesenchyme provides the connective tissue envelopment, vasculature including fenestrated capillaries and lymphatics; it is also a route for vasomotor nerves.



Ultimobranchial Body


Already noted as an endodermal diverticular part of the *caudal pharyngeal complex* (3.70^[7]), it separates from the ectoderm of the fourth pharyngeal cleft and loses its connection with the pharynx by attenuation and rupture of the common pharyngobranchial duct. It becomes closely associated with the expanding lateral lobe of the thyroid gland, and the superior parathyroid (parathyroid IV) component of the complex lying dorsally and outside the thyroid gland. The remainder of the complex, which includes the ultimobranchial body and possibly some vestiges of the ventral recess of the fourth pharyngeal pouch and of the transitory fifth pharyngeal pouch, is enveloped by the thyroid gland. Although some controversy reigned, it is now strongly supported by evidence that the cells of the ultimobranchial body give rise to the 'C' or parafollicular cells producing calcitonin in the thyroid gland of many if not all mammals (Halmi 1986^[8]). Calcitonin has been isolated from ultimobranchial tissue in vertebrates other than mammals (Copp et al 1967^[9]; Taylor 1968^[10]). The derivation of thyroid parafollicular cells has now been clearly demonstrated in embryonic sheep (Jordan et al 1973^[11]).


Pharynx

Study of the development of the head region has so far focused on local segmentation and the integration of nerves with neural crest derived connective tissue and paraxial mesenchyme, or somite, derived voluntary muscle. However, the mechanisms of formation of the pharynx, the role of the endoderm, and the specification of the junction between voluntary with involuntary muscle, are intimately related to the above and have still to be addressed, adding an increased level of complexity to the problem. The pharyngeal endoderm is in contact with mesenchyme

and epithelia from many different sources e.g. neural crest, paraxial mesenchyme of the somitomeres, somites, lateral plate mesenchyme, which at this level is unsplit, cleft ectoderm, general endothelium and the outflow tract of the heart. The development of this region is likely then to be an interaction of all of these tissues in concert. The mechanism of formation of the pharynx is complex and intimately related to the development of the viscerocranium and laryngeal cartilages (p. 274 ). The inter-related roles of endoderm, neural crest, unsegmented paraxial mesenchyme, somites and splanchnopleuric mesenchyme in this region are not yet clear. It is likely that local development is controlled by *Hox* gene expression as seen in viscerocranial development (see 3.148 ).

Whereas the distal foregut, midgut and hindgut are formed from three layers: a serous or adventitial layer, a layer of splanchnopleuric mesenchyme derived from the splanchnopleuric coelomic epithelium, and an inner endodermal epithelium, the proximal foregut has a mix of voluntary muscle around the upper pharynx blending, over the middle third of the oesophagus, into involuntary muscle of the lower oesophagus. The interface between the voluntary pharyngeal muscles and the gut involuntary muscles has not yet been clearly elucidated. A new mesenchymal population has been identified at the interface between the endoderm and the paraxial mesenchyme of the somitomeres and occipital somites (Noden 1991 ); it develops in a rostrocaudal and lateromedial sequence. Beginning as a sparse layer, it becomes denser prior to the formation of endothelial networks, ultimately forming a fenestrated mesenchymal monolayer between developing blood vessels and the endoderm. Later it expands between the notochord and the roof of the foregut, and, it is suggested (Noden 1991 ), that it participates in the formation of pharyngeal and oesophageal smooth muscle and connective tissues. Further experimental studies are needed to confirm this; generally there is much in this region which requires extensive study.

As well as the pouch development described above, the endodermal aspect of the first (maxillomandibular) arch in its dorsal part contributes to the formation of the lateral wall of the nasopharynx in front of the orifice of the auditory tube. The ventral end of the first pouch becomes obliterated, but its dorsal end persists and deepens as the head enlarges. It remains close to the ectoderm of the dorsal end of the first cleft (see above) and, together with the adjoining lateral part of the pharynx and dorsal part of the second pharyngeal pouch, constitutes the *tubotympanic recess*, which forms the tympanic cavity and the auditory tube (p. 1370 ), and ultimately their extensions. The site of the second arch is partly indicated by the *palatoglossal arch*, but its dorsal end is separated from its ventral end by the forward growth of the third arch, which obliterates the intermediate part. Some believe that the site of the second pharyngeal pouch is represented by the *intratonsillar cleft*, around which the tonsil is developed. The third arch forms the *lateral glosso-epiglottic fold*, and its dorsal end takes part in the formation of the floor of the auditory tube. The ventral ends of the fourth arches fuse with the caudal part of the hypobranchial eminence and so contribute to the formation of the *epiglottis*. The adjoining portion becomes connected to the *arytenoid swelling* and may be identified in the *aryepiglottic fold*.

After the caudal part of the hypobranchial eminence has separated from the pharyngeal (posterior) part of the tongue (p. 175 ), it is in continuity with two linear ridges which appear

in the ventral wall of the pharynx, the whole forming an inverted U, sometimes regarded as an independent formation, the *furcula* (of His). These vertical ridges have been identified as the sixth arches, placed very obliquely owing to the shortness of the pharyngeal floor compared with the greater extent of the roof. The ridges of the furcula are carried downwards on the ventral wall of the foregut and bound the median *laryngotracheal groove*, from which the lower part of the larynx, the trachea, bronchi and lungs are developed (see below). At the cranial end of the groove, paired arytenoid swellings arise which convert the slit-like upper aperture of the respiratory system into a T-shaped opening. The aryepiglottic folds (fourth arch derivatives) can be recognized at this stage.

Respiratory System

The development of the respiratory tree begins at stage 12 (approximately 26 days) when there is a sharp onset of epithelial proliferation within the foregut at regions of the endoderm tube destined to become the lungs, stomach, liver and dorsal pancreas (O'Rahilly & Muller 1986^[4]). The future *respiratory epithelium* bulges ventrally into the investing splanchnopleuric mesenchyme then grows caudally as a bulb-shaped tube (3.71^[5]). By stage 13 the caudal end of the tube has divided asymmetrically forming the future primary bronchi; with growth the right primary bronchus becomes orientated more caudally whereas the left extends more transversely. The *trachea* is clearly recognizable at stage 14. From this time the origin of the trachea remains close to its site of evagination from the future oesophagus, however, longitudinal growth of the trachea causes the region of the future carina to descend. Failure of separation of the trachea and oesophagus results in a *tracheo-oesophageal fistula* connecting one tube to the other. The condition also occurs if there is excessive ventral displacement of the dorsal wall of the foregut. This may result in an upper oesophageal segment which is separated from a thin distal tracheo-oesophageal fistule. The latter is usually in continuity with the lower oesophageal segment. Infants with this condition may appear to salivate excessively at birth, with or without respiratory distress (Beasley & Myers 1994^[6]).

Larynx

Formed from the cranial end of the respiratory diverticulum, the laryngotracheal groove, the larynx is bounded ventrally by the caudal part of the hypobranchial eminence (p. 175^[7]) and on each side by the ventral ends of the sixth arches. In the latter, two *arytenoid swellings* appear, one on each side of the groove (3.71A^[8], 3.71B^[9]), and as they enlarge they approximate to each other and to the caudal part of the hypobranchial eminence (3.71A^[10], 3.71B^[11]) from which the *epiglottis* is developed. The *opening* into the larynx, at first a vertical slit, is converted into a T-shaped cleft by the enlargement of the arytenoid swellings; the vertical limb of the T lies between the two swellings and its horizontal limb between them and the epiglottis. The arytenoid swellings differentiate into the *arytenoid* and *corniculate cartilages*, and the ridges joining them to the epiglottis become the definitive *aryepiglottic folds* in which the *cuneiform cartilages* are derived from the epiglottis. The *thyroid cartilage* is developed from the ventral ends of the cartilages of the fourth, or fourth and fifth, pharyngeal arches; it appears as two lateral plates, each chondrified from two centres and united in the midventral line by a fibrous membrane in

which an additional centre of chondrification develops. The *cricoid cartilage* arises from two cartilaginous centres, which soon unite ventrally, gradually extend and ultimately fuse on the dorsal surface of the tube as the cricoid lamina (see also p. 1638). For literature on the early development of the larynx, consult O'Rahilly and Tucker (1973).

Right and Left Lung Buds

These grow dorsally, passing each side of the relatively smaller oesophagus and bulging into the laterally situated pericardio-peritoneal canals (3.67, 71). The parts of the latter accommodating the early lung buds may now be designated the *primary or primitive pleural coeloms*. The dramatic morphogenetic events whereby the primary pleural coelom and its contained developing lung, on each side, excavates and expands into the somatopleuric coelomic epithelium and mesenchyme, thus 'splitting' the primitive body wall into superficial and deep laminae, and forming the extensive *secondary or definitive pleural cavity* is considered further on page 180.

The investing mesenchyme surrounding the lung buds contains a mixed population of cells, some destined to pattern endodermal epithelium and others to produce the endothelial network which will surround the future airsacs; further mesenchymal cells will differentiate as the smooth muscle cells which surround both the respiratory tubes and the blood vessels. In stage 13 embryos, proliferation of the adjacent *splanchnopleuric coelomic epithelium* (of the primary pleural cavities) provides the *investing mesenchyme* which envelops the developing trachea and lung buds. The proliferative activity decreases in stage 14 and the mesenchyme becomes arranged in zones around the developing endoderm. At stage 15, angiogenetic mesenchyme is apparent around the primary bronchi; it forms an extensive capillary network around each lung bud, receiving blood from the developing sixth aortic arch artery and draining it into an anastomosis connected to the dorsal surface of the left atrium. After this stage the coelomic epithelium at the perimeter of the lung surface follows a differentiation pathway to form the *visceral pleura*.

At stage 15 differences in the histology of the oesophagus and the trachea can be seen. The oesophagus has a developing submucosa and muscular coats whereas the larger trachea has a connective tissue coat containing chondroblasts. The *lobar or secondary bronchi* can be seen at stage 16 and the *bronchopulmonary segments* are present at stage 17 when the lung enters the pseudoglandular phase of development. The trachea and oesophagus separate during stage 17.

Later stages of respiratory development see the repeated division of the bronchial tree to form the subsegmental bronchi. Stage 17 correlates to approximately 7 weeks when the embryo has achieved a crown rump length of about 12+ mm. The development of the lungs now moves into the fetal period (stage 23) and continues into the neonatal and postnatal periods. Various stages of lung development have been identified on the basis of the histological appearance of the lungs. They are:

- the pseudoglandular stage, from 7–17 weeks, when the lung resembles a

tubulo-acinar gland

- the canalicular stage from 17–26 weeks
- the saccular stage from 24 weeks to birth
- the alveolar stage which begins before birth and continues into childhood perhaps up to 8 years of age although the time of completion of alveolar formation has not yet been established.

Pseudoglandular Stage

This stage is described as extending approximately from weeks 7–17; it covers the development of the lower conducting airways and the appearance of the acinar structures. The growth and branching of the endoderm epithelium is controlled by the local investing splanchnopleuric mesenchyme as in most epithelial/mesenchymal interactions. The airways begin to differentiate during this stage, being lined proximally by high columnar epithelium and distally by cuboidal. Later the upper airways are lined with pseudostratified epithelium. Mucous glands develop by the 12th week and enlarge in the submucosa; secretory activity has been identified in the trachea at 14 weeks (Bucher & Reid 1961^[9]). The splanchnopleuric mesenchyme condenses around the epithelium and differentiates into smooth muscle and connective tissue cell types. Cartilage differentiation in the airways is poorly described; it is not clear if cartilage is synthesized in the pseudoglandular stage.

Canalicular Stage (17–26 Weeks)

During this phase there are about three generations of branching after which the mesenchyme around the branching tips of the dividing respiratory tree decreases allowing the distal airspaces to widen. At 23 weeks longitudinal sections of the future distal regions show a sawtooth margin which may indicate the site of further acini. Peripheral growth is accompanied by an increase in the capillary network around the distal airspaces where, in many places, close contact is made with the respiratory cuboidal epithelium. At such contacts the respiratory epithelial cells decrease in height and begin to differentiate as type I pneumocytes. The cells which remain cuboidal are type II pneumocytes, which are believed to be the stem cells of the alveolar epithelium; they develop an increasing number of lamellar bodies which store surfactant from 6 months of gestation. With apposition of the capillary networks to the thin pneumocytes, and with reduction of the interstitial tissue of the lung gas exchange becomes possible.

Saccular Stage (24 Weeks to Birth)

At this stage thin walled terminal saccules are apparent, which will become alveolar ducts as development proceeds. There is a tremendous expansion of the prospective respiratory airspaces during this period which leads to a decrease in the interstitial tissue. The capillary networks become closely opposed as the airspaces get closer together. Invaginations termed secondary crests develop from the saccule walls. As a crest protrudes into a saccule, part of the capillary network becomes drawn in it. After the later expansion of the saccules on each side of the crest,

a double capillary layer becomes annexed between the now alveolar walls. During the saccular stage elastin is deposited beneath the epithelium, an important step for future alveolar formation. The production of surfactant matures during this stage increasing the chances of the fetus to survive should it be born prematurely.

Alveolar Stage

Exactly when the saccular structure of the lung can be termed alveolar is not yet clear, different workers having different definitions of what constitutes an alveolus. Thurlbeck (1992^[1]) notes that alveoli can be seen at 32 weeks and are present in all fetuses at 36 weeks which he recommends as the beginning of the alveolar stage, whereas Hislop et al (1986^[2]) suggested that the stage should commence at 28 weeks. As the distal airspaces expand during late gestation and continue after birth, there is a process of fusion of the capillary nets from one alveolus to the adjacent alveolus. Thus shortly after birth there is an extensive double capillary net. Fusion of these layers is apparent at 28 days postnatally and extensive at 1.5 years; it is probably complete by 5 years.

Interactions of Early Respiratory Development

The control of the branching pattern of the respiratory tree resides with the *splanchnopleuric mesenchyme*. Recombination of tracheal mesenchyme with bronchial respiratory endoderm results in inhibition of bronchial branching, whereas recombination of bronchial mesenchyme with tracheal epithelium will induce bronchial outgrowths from the trachea (Wessels 1971^[3]; Hilfer et al 1985^[4]). Initially the tracheal mesenchyme is continuous with that surrounding the ventral wall of the oesophagus, but with lengthening and division of the tracheal bud and deviation of the lung buds dorsally, each bud becomes surrounded by its own specific mesenchyme thus permitting regional differences between the lungs, i.e. the number of lobes, or the degree of growth and maturity of a particular lung. Each lung develops by a process of dichotomous branching. For branching to occur a cleft must develop in the tip (or side) of the epithelial tube. The epithelium then evaginates each side of the cleft forming new branches which lengthen; the process is then repeated. Differences have been noted between the mesenchyme closely associated with the endoderm epithelium and that some distance away. At the tips of the developing epithelial buds the mesenchyme is flattened and densely packed; in contrast, along the side of the bud and in the clefts the mesenchyme forms an ordered row of cuboidal cells. Cells in both arrangements send processes towards the epithelial basal lamina which is thicker in the clefts, but so thinned as to be almost indistinguishable on the tips of the buds where the epithelium and mesenchymal cells form intimate contacts. Tenascin, an extracellular matrix molecule (also known as hexabrachion or cytotactin), is present in the budding and distal tip regions, but absent in the clefts. Conversely fibronectin, an extracellular matrix molecule found commonly in basal laminae, is found in the clefts and along the sides of the developing bronchi, but not on the budding and distal tips (Abbott et al 1992^[5]; see also p. 114^[6]).

In extensive studies on lung development in mammals Ten Have-Opbroek (1991^[7]) has disputed that the pseudoglandular stage covers the development of the complete bronchial tree. He and his

co-workers maintain that lung development can be divided into causally distinct bronchial and respiratory systems both of which proceed in the canalicular, saccular and alveolar stages. The epithelium of the developing bronchial system is columnar whereas in the respiratory system it is cuboidal. This latter epithelium is composed of precursors of the type II pneumocytes which exhibit early stages of multilamellar bodies. There is always a sharp demarcation between alveolar epithelium and bronchial epithelium throughout development, leading these workers to postulate that the bronchial and respiratory systems each originate from a different portion of the primordial respiratory diverticulum. The type II pneumocyte is the key cell in pulmonary acinus formation being the stem cell which produced type I pneumocytes and which ultimately matures into a surfactant producing cell. The cells at the distal end of the bronchial system are non-ciliated Clara cells which develop slowly prenatally.

Mesenchyme around the lung buds may be destined to become:

- the interstitial connective tissue of the lung
- endothelial networks (both blood vascular and lymphatic) and blood cells of the pulmonary and bronchial circulations
- smooth muscle cells which surround either the airways or the blood vessels.

Of the connective tissues line, lung fibroblasts retain an influence over the rate of cytodifferentiation and maturity of the lung epithelium. Lung fibroblasts from the pseudoglandular stage stimulate epithelial cell proliferation, whereas fibroblasts from the saccular stage promote differentiation (Caniggia et al 1991^[5]). From the saccular stage lung fibroblasts secrete an oligopeptide, fibroblast-pneumocyte factor (FPF), which stimulates neighbouring type II pneumocytes in the developing alveolar walls to produce the surfactant phospholipid, saturated phosphatidylcholine (SPC) contained in multilamellar bodies. In recent years premature babies and those who will be delivered preterm have been given cortisol, which binds to specific receptors in lung mesenchyme causing release of FPF, thus accelerating lung maturity.

There is an interesting sexual dimorphism in lung development. Androgens have been found to delay fetal lung maturation while stimulating fetal lung growth. Male type II cells are less mature than the female cells and this is thought to be due to delayed fibroblast maturation caused by androgens blocking the cortisol stimulation of FPFm RNA (Torday 1992^[6]).


The development of lung smooth muscle has been demonstrated by the use of antibodies against cytoskeletal and contractile proteins (Mitchell et al 1990^[7]). Initially the local lung mesenchyme is positive for vimentin filaments; however, this is later replaced by desmin in cells destined to become smooth muscle. The expression of both desmin and smooth muscle myosin indicates terminal differentiation of smooth muscle; however, α -smooth muscle actin-containing cells, which form a thick coat around the primitive airways, have been found to extend further than either the desmin or smooth muscle myosin-containing cells. It was noted that α -actin positive cells were found in regions of epithelial cleft formation, suggesting an association with



branching morphogenesis.

Endothelial development is seen in the pseudoglandular stage when capillary networks form around the developing lung buds. These will become the capillary anastomoses around the future alveoli. The mesenchyme produces both the endothelium of the vessel tunica intima and the smooth muscle cells of the tunica media. Vimentin is noted in the smooth muscle cells around developing vessels in the pseudoglandular stage but this is replaced by desmin in the sacular stage.



Thoracic Wall and Pleural Cavities


Whereas the preceding account describes the morphological and histological development of the respiratory tree, for the lungs to function they must be surrounded by a complete pleural cavity slightly larger than the capacity of the lungs. The development of the thoracic cage and the pleural cavities is therefore of vital importance for the functioning of the respiratory system.


At the same time as the splanchnopleuric mesenchyme is being produced from the proliferating coelomic epithelium so too is the somatopleuric. This latter mesenchyme is penetrated by the developing *ribs* which arise from the thoracic sclerotomes. In the midline the somatopleuric mesenchyme gives rise to the *sternum* and *costal cartilages* (see p. 538 ). The bony and cartilaginous cage provides insertions for the intercostal muscles which arise from the ventrolateral edge of the epithelial plate of the somites. The somatopleuric coelom epithelium after its proliferative phase gives rise to the parietal layer of pleura.

When the lung buds develop they project into the *pericardio-peritoneal canals* subdividing them into *primary pleural coeloms* around the lung beds cranially, and paired *peritoneal coeloms* caudally which are continuous with the wider peritoneal coelom around the mid- and hindgut. The communications with the pericardial and peritoneal coeloms become the *pleuropericardial* and *pleuroperitoneal canals* respectively (3.72 , 78 ). (When separation between these fluid-filled major coelomic regions is advancing towards completion, they are named the *pericardial*, *pleural* and *peritoneal cavities*; the serous walls of the latter are often called *sacs*. In early embryos the cavities retain substantial volumes of fluid and their walls are separate; they provide the route for a primitive type of circulation until superseded by the blood vascular system. In later fetal and postnatal life cavity walls are coapted, a mere microscopic film of serous fluid intervening.)



A curved elevation of tissue, the *pulmonary ridge*, develops on the lateral wall of the pleural coelom and partly encircles the pleuropericardial canal. The ridge is continuous with the dorsolateral edge of the septum transversum. The developing lung bud abuts on the ridge, which as a result divides into two diverging membranes meeting at the septum transversum. One is cranially placed and termed the *pleuropericardial membrane*; embedded within it the common cardinal vein and phrenic nerve reach the septum transversum by this route. The other membrane, caudally placed, is termed the *pleuroperitoneal membrane*. As the apical part of the lung forms it invades and **splits** the body wall and extends cranially on the **lateral aspect** of the

common cardinal vein, carrying with it, or rather preceded by, an extension from the primary pleural coelom to form part of the *secondary* or *definitive pleural sac*. In this way the common cardinal vein and the phrenic nerve come to lie medially in the mediastinum. The pleuropericardial canal, which lies medial to the vessel, is gradually narrowed to a slit, which is soon obliterated by the apposition and fusion of its margins (3.72 ). Its closure occurs early and is mainly effected by the growth and expansion of the surrounding viscera, heart and great vessels, lungs, trachea and oesophagus, and not by active growth of the pleuropericardial membrane across the opening to the root of the lung (3.72 ).




In addition to its extension in a cranial direction the lung and its associated visceral and parietal pleura also enlarge ventromedially and caudodorsally (see below). With the ventromedial extension, the lungs and pleurae therefore excavate and split the somatopleuric mesenchyme over the pericardium, separating the latter from the ventral and lateral thoracic walls (3.73 ). Thus the ventrolateral fibrous pericardium, parietal serous pericardium and mediastinal parietal pleura, although topographically deep, are *somatopleuric in origin*.

Separation of pleural and peritoneal cavities is effected by development of the diaphragm. The *septum transversum* is at first a condensation of mesenchyme, caudal to the pericardial cavity and extending from the ventral and lateral regions of the body wall to the foregut. Dorsal to it on each side is the relatively narrow *pleuroperitoneal canal*. The endodermal hepatic bud grows into the septum transversum, which then can be seen to consist of two parts. One, the *pars diaphragmatica*, is disposed in the transverse plane and lies over the convex cranial surface of the putative liver. The other, the *pars mesenterica*, lies initially in the median sagittal plane and is expanded by the developing liver. At this stage the liver is widely attached to the *pars diaphragmatica* and to the ventral abdominal wall. These attachments are the forerunners of the coronary and triangular ligaments and of the falciform ligament respectively. Medial to the pleuroperitoneal canals are the oesophagus and stomach with their dorsal mesentery, and, at the root of the latter, the dorsal aorta. Dorsolateral to the canals are the pleuroperitoneal membranes, which remain small; dorsally are the mesonephric ridges, suprarenals and gonads. Just as the enlargement of the pleural cavity cranially and ventrally is effected by a process of burrowing into the body wall, so its caudodorsal enlargement is effected in the same way. The expanding pleural cavities extend into the mesenchyme *dorsal* to the suprarenal glands, the gonads and (degenerating) mesonephric ridges. Thus somatopleuric mesenchyme is peeled off the dorsal body wall to form a substantial portion of the dorsolumbar part of the diaphragm. The pleuroperitoneal canal is closed by the fusion of its edges, which are carried together by growth of the organs surrounding it and, in particular, that of the suprarenal, which carries the dorsal margin of the canal ventrally to meet the *pars diaphragmatica* of the septum transversum (Wells 1954 ). The right pleuroperitoneal canal closes earlier than the left. Hence it is on the left that an abnormal communication persisting between the pleural and peritoneal cavities is encountered more frequently.

While these changes occur, the septum transversum undergoes a progressive alteration in relative position. In a 2-mm human embryo, the dorsal border of the septum transversum lies opposite the second cervical segment but, as the embryo grows and the heart enlarges, it migrates caudally. At first the ventral border moves more rapidly than the dorsal, but after the embryo has

attained a length of 5 mm it is the dorsal border which migrates more rapidly (3.112 ). When the dorsal border of the septum transversum lies opposite the fourth cervical segment, the phrenic nerve (C3, 4 and 5) and portions of the corresponding myotomes grow into it and accompany it in its later migrations. It is not until the end of the second month that the dorsal border of the septum transversum is opposite the last thoracic and first lumbar segments, the final position occupied by some of the dorsal attachments of the diaphragm and some derivatives of the pars mesenterica. However, the main derivatives of the pars diaphragmatica lie at considerably more cranial levels (see below and p. 270 .

Formation of the Diaphragm

The closure of the pleuroperitoneal openings completes a mainly mesenchymal partition which thereafter separates thoracic from abdominal viscera and forms the framework for the future diaphragm. This has a composite origin from many different mesenchyme sources. The sternal and costal parts are derived almost exclusively from the pars diaphragmatica of the septum transversum mesenchyme, with a small dorsolateral contribution from the pleuroperitoneal membranes and by excavation of the somatopleuric mesenchyme of the thoracic wall (costal part). Anterior to the oesophageal hiatus is a small contribution from the cranial oesophagophrenic continuation of the gastrohepatic part of the lesser omentum, both derived from the pars mesenterica of the septum transversum. Between the oesophageal and aortic hiatuses it is formed by the dorsal mesentery (strictly the dorsal meso-oesophagus but often, less precisely, included as part of the dorsal mesogastrium). The remainder of the lumbar part of the diaphragm is formed from mesenchyme around the abdominal aorta and more laterally from somatopleuric mesenchyme of the dorsal body wall behind the suprarenal, mesonephric ridge and gonad (Wells 1954 ). Some authorities consider that much greater areas of the adult diaphragm are derived from the pleuroperitoneal membranes and from the chest wall. Gaps between the lumbar and costal parts of the diaphragm are usually due to underdevelopment of the latter. Premitotic myoblasts, derived principally from the ventrolateral edges of the fourth cervical somites, invade the septum transversum (described on p. 270 ). They extend throughout the mesenchymal partition, giving rise to the muscular diaphragm (3.135 ) and its fibrous central tendon, or aponeurosis, with its trefoil shape, cruciform intersecting fibres and central nodal thickening. The caudal migration of the diaphragm during development has already been described (see above).

Maturation of the Lungs

Whereas many fetal organs are able to grow to normal proportions even if they are in abnormal locations this is not the case for the lungs. Lung growth becomes impaired by restricted expansion and it is suggested that distension of the developing lung may provide a major stimulus to growth. Absence or impairment of fetal breathing movements is associated with pulmonary hypoplasia, as are defects affecting diaphragmatic activity. It is believed that normal fetal breathing movements increase the lung volume and stimulate growth of the distal airspaces. The mucous glands of the trachea and bronchi secrete a lung fluid during development. This fluid usually passes up the respiratory tract to mix with the amniotic fluid. Experimental ligation of the trachea results in accumulation of the fluid with the lung becoming much heavier than

normal.

The relationship between lung fluid and amniotic fluid is far more complex than was previously thought. Pulmonary hypoplasia at birth is often associated with severe congenital urinary obstruction, as in Potter's syndrome. Thus there is a developmental link between development of the lungs and the kidneys. In renal agenesis there is reduced bronchial branching as early as 12–14 weeks of gestation, before amniotic fluid is produced by the kidneys, suggesting a direct renal factor which supports lung development (Peters et al 1991^[4]). Later, the presence of amniotic fluid is necessary for normal fetal lung development. The fetal lung is a net fluid secretor; most of the fluid produced remains within the lungs as a mechanical effect of the amniotic fluid pressure; normally only a small amount of this fluid contributes to the amniotic fluid. Thus the normal functioning of the kidneys regulates the volume and pressure of the lung airway fluid and may in turn provide the pressure needed for expansion and enlargement of the bronchial and pulmonary systems. Interestingly, prolonged experimental lung drainage accelerates the maturity of the alveolar cells, possibly due to an inappropriate signal that birth is imminent (see also pp. 204^[4], 335^[4].)

Postpharyngeal Foregut

Although the postdiaphragmatic gut is subdivided into three embryological portions, fore- mid- and hindgut, there are no corresponding fundamental morphological and cytological distinctions between the three parts. Thus the foregut produces a portion of the duodenum as does the midgut, and the midgut similarly produces large intestine as does the hindgut. The differences between portions of the gut develop as a result of interactions between the three embryonic tissue layers which give rise to the gut, namely:

- the endodermal inner epithelium
- the thick layer of splanchnopleuric mesenchyme
- the outer layer of proliferating splanchnopleuric coelomic epithelium.



The final layers of the gastrointestinal tract are derived as follows:


- The epithelial layer of the mucosa and connected ducts and glands are from the endodermal epithelium.
- The lamina propria and muscularis mucosa, the connective tissue of the submucosa, the muscularis externa and the external connective tissue are all from the splanchnopleuric mesenchyme.
- The outer peritoneal epithelium is from the splanchnopleuric coelomic epithelium.

Blood vessels and lymphatics develop from local populations of angiogenic mesenchyme throughout the gut, as do lymph nodes. Innervation of the gut is via the neural crest derived enteric and autonomic systems of nerves and plexuses. There is a craniocaudal developmental











gradient along the gut with the stomach and small intestine developing in advance of the colon.

Oesophagus






The oesophagus can be distinguished from the stomach at stage 13 (embryo 5 mm). It elongates during successive stages and its absolute length increases more rapidly than the embryo as a whole. Cranially it is invested by splanchnopleuric mesenchyme posterior to the developing trachea, and more caudally between the developing lungs and pericardio-peritoneal canals posterior to the pericardium. Caudal to the pericardium, the terminal, pregastric segment of the oesophagus has a short thick *dorsal meso-oesophagus* (from splanchnopleuric mesenchyme), while ventrally it is enclosed in the cranial stratum of the septum transversum mesenchyme (i.e. a short *ventral meso-oesophagus*). Each of the above are continuous caudally with their respective primitive dorsal and ventral mesogastra (see p. 186 ). Thus the oesophagus has only limited areas related to a *primary* coelomic epithelium. However, note the subsequent development of the para-oesophageal right and left pneumato-enteric recesses (3.79 ), the relation of the ventral aspect of the middle third of the oesophagus to the oblique sinus of the pericardium, and the relation of its lateral walls in the lower thorax to the mediastinal pleura. All the foregoing are *secondary extensions* from the primary coelom.


The mucosa consists of two layers of cells by stage 15 (week 5), but the proliferation of the mucosa does not occlude the lumen at any time. The mucosa becomes ciliated at 10 weeks, and stratified squamous epithelium at the end of the 5th month; occasionally patches of ciliated epithelium may be present at birth. Circular muscle can be seen at stage 15 but longitudinal muscle has not been identified until stage 21. Neuroblasts can be demonstrated in the early stages; the myenteric plexuses have cholinesterase activity by 9.5 weeks and ganglion cells are differentiated by 13 weeks. It is suggested that the oesophagus is capable of peristalsis in the first trimester (Smith & Taylor 1972 ). Peristalsis along the oesophagus and at the lower oesophageal sphincter is immature at birth resulting in frequent regurgitation of food in the newborn period. The pressure at the lower oesophageal sphincter approaches that of the adult at 3–6 weeks of age.

Stomach

At the end of the fourth and beginning of the fifth week the stomach can be recognized as a fusiform dilation (3.74 , 77 ) cranial to the wide opening of the midgut into the yolk sac. By the fifth week this opening has narrowed into a tubular vitelline intestinal duct (3.74 , 75 , 76 ), which soon loses its connection with the digestive tube (3.76 ). At this stage the stomach is median in position and separated cranially from the pericardium by the septum transversum (p. 151 ), which extends caudally on to the cranial side of the vitelline intestinal duct and ventrally to the somatopleure. Dorsally, the stomach is related to the aorta and, reflecting the presence of the pleuroperitoneal canals on each side, is connected to the body wall by a short dorsal mesentery, the *dorsal mesogastrium* (3.79 ). The latter is directly continuous with the dorsal mesentery (mesenteron) of almost all of the remainder (except its caudal short segment) of the intestine. The liver develops as a hollow outgrowth from the ventral aspect of the foregut and grows cranially into the substance of the septum transversum (3.74 , 76 ),

this part of the septum (pars mesenterica) now being termed the ventral mesogastrium. The rest of the intestine has no ventral mesentery.

In human embryos of 10 mm, the characteristic gastric curvatures are already recognizable. (What follows in this paragraph is largely a summary of long-held traditional views of gastric and omental development. More recently close reappraisal of human embryonic serial sections has led to many major descriptive changes and alternative proposals, see p. 192 ) Growth is more active along the dorsal border of the viscus; its convexity markedly increases and the rudimentary fundus appears. Because of more rapid growth of the dorsal border, the pyloric end of the stomach turns ventrally and the concave lesser curvature becomes apparent (3.76 ). The stomach is now **displaced** to the left of the median plane and apparently becomes physically **rotated**; thus its original right surface becomes dorsal and its left ventral. Accordingly the right vagus nerve is distributed mainly to the dorsal and the left mainly to the ventral surface of the organ. The dorsal mesogastrium increases in depth and becomes folded on itself; the ventral mesogastrium becomes more coronal than sagittal. The pancreatoco-enteric recess (p. 186 ) hitherto usually described as a simple depression on the right side of the dorsal mesogastrium, becomes dorsal to the stomach and excavates downwards and to the left between the folded layers. It may now be termed the inferior recess of the *bursa omentalis*. Thus the stomach may be simplistically described as having two rotations. The first 90° clockwise, viewed from the cranial end, the second 90° clockwise about an anteroposterior axis. The displacement, morphological changes and apparent 'rotation' of the stomach have been attributed variously to its own and surrounding differential growth changes, extension of the pancreatoco-enteric recess with changes in its mesenchymal walls, and pressure, particularly by the rapidly growing liver (Kanagasuntheram 1957 ). (As intimated, the account just given prevails in basic courses and textbooks and perhaps suffices for some purposes. For a more complete treatment see p. 192  et seq.)

Mucosal and submucosal development can be seen in the 8th to 9th weeks. No villi form in the stomach, unlike other regions of the gut, instead glandular pits can be seen in the body and fundus. These develop in the pylorus and cardia by weeks 10 and 11 when parietal cells can be demonstrated. *Acid secretion* has not been demonstrated in the fetal stomach before 32-week gestation, however *intrinsic factor* has been detected after 11 weeks. This increases from the 14th to 25th week until the pylorus, which contains a larger number of parietal cells than in the adult, also contains a relatively larger quantity of intrinsic factor. The significance of the early production of intrinsic factor and the late production of acid by the parietal cells is not known. Chief cells can be identified after weeks 12–13, although they cannot be demonstrated to contain pepsinogen until term. Also over the same period mucous neck cells can be seen which actively produce mucus from week 16. *Gastrin* producing cells have been demonstrated in the antrum between 19 and 20 weeks and gastrin levels have been measured in cord blood and in the plasma at term. Interestingly cord serum contains gastrin levels 2–3 times higher than those in maternal serum. (For an extensive account of gastric development see Grand et al 1976 .)

The stomach muscularis externa develops its circular layer at 8–9 weeks when neural plexuses also develop in the body and fundus. The longitudinal muscle develops slightly later. The pyloric musculature is thicker than the rest of the stomach, although generally the thickness of the total

musculature of the stomach at term is reduced compared to the adult.

The serosa of the stomach derives from the splanchnopleuric coelomic epithelium (see above). No part of this serosa undergoes absorption; the original left side of the stomach serosa faces the greater sac, the right side the lesser sac (see below).



Duodenum

The duodenum is divided into four parts in the adult for descriptive purposes. From an embryological viewpoint the duodenum forms the caudal part of the foregut and the cranial part of the midgut. The importance of such a distinction lies with the presence of a ventral mesoduodenum (continuous cranially with the ventral mesogastrium), which is attached only to the foregut portion, and the derivation of the pancreas and liver, which from their origin are foregut structures.

Posteriorly the duodenum has a thick *dorsal mesoduodenum* which is continuous with the dorsal mesogastrium cranially and the dorsal mesentery of the midgut caudally. Anteriorly the extreme caudal edge of the ventral mesentery of the foregut extends onto the short initial segment of the duodenum. The liver arises as a diverticulum from the ventral surface of the duodenum at the foregut–midgut junction, i.e. initially where the midgut is continuous with the yolk sac wall (i.e. the cranial intestinal portal); the ventral pancreatic bud also arises from this diverticulum. The dorsal pancreatic bud evaginates posteriorly into the dorsal mesoduodenum slightly more cranially than the hepatic diverticulum. The 'rotations', differential growth, and cavitations related to the stomach and omenta cause corresponding movements in the duodenum which forms a duodenal loop directed to the right, with its original right side now adjacent to the posterior abdominal wall. This shift is compounded by the migration of the bile duct and ventral pancreatic duct around the duodenal wall; their origin shifts until it is found in the medial wall of the second part of the fully formed duodenum; the bile duct passes posteriorly to the duodenum and travels in the free edge of the ventral duodenum and ventral mesogastrium. Local adherence and then absorption of part of the duodenal serosa and the parietal peritoneum results in almost the whole of the duodenum becoming retroperitoneal apart from a short initial segment.




Dorsal and Ventral Mesenteries of the Foregut

It is important to realize that the epithelium of the stomach and duodenum does not rotate relative to its investing mesenchyme. The rotation *includes* the coelomic epithelial walls of the pericardio-peritoneal canals, which are each side of the stomach and duodenum forming its serosa, and the elongating dorsal mesogastrium or much shorter dorsal mesoduodenum. A *ventral mesogastrium* can be seen when the distance between the stomach and liver increases. Whereas the *dorsal mesogastrium* takes origin from the posterior body wall in the midline, its connection to the greater curvature of the stomach, which lengthens as the stomach grows, becomes directed to the left as the stomach undergoes its first rotation. With the second rotation a portion of the dorsal mesogastrium now faces caudally. The ventral mesogastrium remains as a double layer of coelomic epithelium enclosing mesenchyme; this forms the *lesser omentum* (3.79

; see below). Associated with the movement of the stomach is an extensive lengthening of the dorsal mesogastrium which becomes the *greater omentum*. This now, from its posterior origin, droops caudally over the small intestine then folds back anteriorly and ascends to the greater curvature of the stomach. Thus the greater omentum is composed of a fold containing, technically, four layers of peritoneum. The dorsal mesoduodenum is a much thicker structure; it fixes the position of the duodenum when the rest of the midgut and its dorsal mesentery elongate and pass into the umbilical cord. For a more detailed account of this process see page 197 .

Special Glands of the Postpharyngeal Foregut

Pancreas



The pancreas develops from *two* evaginations of the foregut which fuse to form a single organ. A *dorsal pancreatic bud* can be seen in stage 13 embryos as a thickening of the endodermal tube which proliferates into the dorsal mesogastrium (3.77 ). A *ventral pancreatic bud* evaginates in close proximity to the liver primordium and cannot be clearly identified until stage 14 when it appears as an evagination of the bile duct itself. At stage 16 (5 weeks) differential growth of the wall of the duodenum results in movement of the ventral pancreatic bud and the bile duct to the right side and ultimately to a dorsal position. It is not clear whether there is a corresponding shift of mesenchyme during this rotation; however, the ventral pancreatic bud and the bile duct rotate from a position within the ventral mesogastrium (ventral mesoduodenum) to one in the dorsal mesogastrium (dorsal mesoduodenum) which is destined to become fixed onto the posterior abdominal wall. By stage 17 the ventral and dorsal pancreatic buds have fused, although the origin of the ventral bud from the bile duct is still clear. The developing pancreatic ducts usually fuse in such a way that most of the dorsal duct drains into the proximal part of the ventral duct (3.77 ). The proximal portion of the dorsal duct usually persists as an accessory duct. The fusion of the ducts takes place late in development or in the postnatal period: 85% of infants have patent accessory ducts as compared to 40% of adults. Fusion may not occur in 10% of individuals; here separate drainage into the duodenum is maintained (Githens 1989 ). Thus part of the head, the neck, body and tail of the pancreas derive from the dorsal pancreatic bud and the remainder of the head and uncinete process from the ventral bud. During the shift of the ventral bud the superior mesenteric vessels which are extending from the abdominal aorta become trapped between the head and uncinete process of the pancreas. Initially the body of the pancreas extends into the dorsal mesoduodenum and then cranially into the dorsal mesogastrium. As the stomach rotates, this portion of the dorsal mesogastrium is directed to the left forming the posterior wall of the lesser sac. The posterior layer of this portion of dorsal mesogastrium fuses with the parietal layer of the coelom wall (peritoneum) and the pancreas becomes mainly retroperitoneal. The region of fusion of the dorsal mesogastrium does not extend so far left as to include the tail of the pancreas which passes into the lienorenal ligament. The anterior border of the pancreas later provides the main line of attachment for the posterior leaves of the greater omentum.


The evaginations of pancreatic endoderm into the investing mesenchyme become tubular structures which branch progressively. The primitive duct epithelium provides the stem cell

population for all the secretory cells of the pancreas. It gives rise to α cells which produce glucagon, β cells which produce insulin, and δ cells which produce somatostatin during weeks 8–10. Cells containing pancreatic polypeptide (PP) appear somewhat later. Initially these endocrine cells are located in the duct walls or in buds developing from them; later they accumulate in pancreatic islets. The dorsal bud gives rise to mostly α cells and the ventral bud to most of the PP cells. β cells develop from the duct epithelium throughout development and into the neonatal period. Later, in weeks 10–15, some of the primitive ducts differentiate into acinar cells. Zymogen granules or acinar cell markers can be detected at 12–16 weeks. The remaining primitive duct cells will differentiate into definitive ductal cells. In the fetus they develop microvilli and cilia but lack the lateral interdigitations seen in the adult. Branches of the main duct become interlobular ductules which terminate as blind ending acini or as tubular, acinar elements. The connective tissue between the ducts develops from the investing mesenchyme which, in the fetus, appears to be important in stimulating pancreatic proliferation and maintaining the relative proportions of acinar, α and β cells during development.

Liver

The liver is one of the most precocious embryonic organs; it functions as the main centre for haemopoiesis in the fetus. It develops from an endodermal evagination of the foregut and from the septum transversum mesenchyme, a region of unsplit lateral plate mesenchyme in the early embryo which receives mesenchyme cells from the proliferating coelomic epithelium in the protocardiac region. The development of the liver is intimately related to the development of the heart as the vitelline, followed by the umbilical, veins passing to the sinus venosus are disrupted by the septum transversum to form a hepatic plexus the forerunner of the hepatic sinusoids.

The developing liver can first be seen in the stage 11 embryo; the proliferation and bulging of the hepatic diverticulum stimulates the production of blood islands in the investing mesenchyme (3.78 ). By stage 12 the diverticulum has two parts: a caudal part, which will produce the cystic duct and gallbladder, and a cranial part which forms the liver biliary system (see also pancreatic anlage p. 186 ).

Around the cranial portion of the hepatic diverticulum the basal lamina disrupts progressively and individual epithelial cells migrate into the surrounding septum transversum mesenchyme (3.79 ). The previously smooth contour of the diverticulum merges into columnar extensions of endoderm, the so-called epithelial trabeculae, which stimulate the mesenchymal cells to form blood islands and endothelium. The advance of the endodermal epithelial cells promotes the conversion of more and more septum transversum mesenchyme into endothelium and blood cells with only a little remaining to form the scanty (human) liver capsule and interlobular connective tissue. This invasion by the hepatic epithelium is completed in stage 13 when it approaches the caudal surface of the pericardial cavity, with only a thin lamina of mesenchyme intervening which will give rise to part of the diaphragm.

During this early phase of development the liver is far more highly vascularized than the rest of the gut. The hepatic capillary plexus is connected bilaterally with the right and left vitelline veins

and dorsolaterally they empty by multiple channels into enlarged hepatocardiac channels, which lead to the right and left horns of the sinus venosus; usually the channel on the right side is most developed. Both left and right channels bulge into the pericardio-peritoneal canals forming sites for the exchange of fluid from the coelom into the vascular channels (O'Rahilly & Muller 1986). The growth of the hepatic tissue in these regions is sometimes referred to as the left and right horns of the liver.

The liver remains proportionately large during its development forming a sizeable organ dorsal to the heart at stage 14, then more caudally placed by stage 16. By this stage hepatic ducts can be seen separating the hepatic epithelium from the extrahepatic biliary system, but even at stage 17 the ducts do not penetrate far into the liver. Later the lines of endodermal epithelial cells in continuity with the hepatic ducts differentiate into ductal cells following the developmental sequence of connective tissue differentiation around the portal vein and its branches.

The bile duct, which first has origin from the ventral wall of the foregut (now duodenum), migrates with the ventral pancreatic bud first to the right and then dorsomedially into the dorsal mesoduodenum.

As the liver enlarges, it projects more and more into the abdominal cavity. The medial coelomic epithelial walls around the liver constitute much of the ventral mesogastrium (but see below). The mesenchyme between these layers is in continuity with the septum transversum mesenchyme of the diaphragm superiorly. The coelomic epithelial layers of the ventral mesogastrium can almost approximate both anterior and posterior to the liver (a slender lamina of mesenchyme intervening), where they form the falciform ligament and the lesser omentum respectively; they form the visceral peritoneum where they are in contact with the liver. Cranially the liver remains in mesenchymal contact with the diaphragm and the epithelial leaves of the ventral mesogastrium are reflected onto the inferior surface of the diaphragm as the coronary and right and left triangular ligaments. (Strictly, the lesser omentum, in and near its free border, also includes the less extensive ventral mesoduodenum.) It is worth noting that as the ventral body wall develops, the falciform ligament, which attaches to the ventral body wall at the cranial intestinal portal, is drawn to the diminishing cranial rim of the umbilicus. Here it becomes increasingly oblique, curved and indeed 'falciform'. Whereas in the early embryo the connection between one pericardio-peritoneal canal and the other was directly across the ventral surface of the cranial midgut, immediately caudal to the developing primitive ventral mesogastrium, by stage 14 the passage from one side of the falciform ligament to the other necessitates passing below the greatly enlarged liver, or the curved lower edge of the falciform ligament, or lesser omentum. At 3 months the liver almost fills the abdominal cavity and its left lobe is nearly as large as its right. Later when the haematopoietic activity of the liver is assumed by the spleen and bone marrow the relative development of the liver changes and the left lobe actually undergoes some degeneration and becomes smaller than the right. The dominance of the right lobe is reflected in the large expanse of the coronary and right triangular ligaments, compared with the diminutive left triangular ligament (and their respective bare areas). Until birth the liver remains relatively larger than in the adult.

Experimental studies on the epithelial/mesenchymal interactions of liver development have

demonstrated the specificity of the cell populations involved (Le Douarin 1975^[4]). They may be summarized as follows:

- Liver mesenchyme forms the endothelial cells of the liver and the endoderm forms the hepatocytes. If a mechanical barrier is inserted across the mesenchymal hepatic area just caudal to the endodermal outgrowth, liver tissue will develop normally cranial to the barrier where it is in contact with the endoderm; however, caudal to the barrier the mesenchyme will form endothelial cells and hepatic lobes, but there will be no hepatocytes present.
- Hepatic endoderm cells are incapable of differentiating into hepatocytes without hepatic mesenchyme. Cephalic and somitic mesenchyme is unable to promote differentiation of hepatic endoderm.
- Presumptive intestinal endoderm cells combined with hepatic mesenchyme do not produce hepatocytes.
- All derivatives of the lateral plate mesenchyme, both somatopleuric and splanchnopleuric mesenchyme, can promote the differentiation of hepatic endoderm, although not so strongly as hepatic mesenchyme. Lateral plate mesenchyme will form blood sinusoids under these conditions.

It is inferred that matrix or cell surface properties are common throughout the lateral plate mesenchyme but are different from axial mesenchymal cells. Le Douarin (1975^[4]) suggests that the morphogenesis of the liver lobes are patterned by the septum transversum mesenchyme.

Midgut

The midgut forms the third and fourth parts of the duodenum, jejunum, ileum and two-thirds the way along the transverse colon; thus its development results in most of the small and a portion of the large intestine. In embryos of stages 10 and 11 it extends from the cranial to the caudal intestinal portals and communicates directly with the yolk sac over its entire length. Although it has a dorsal wall, at these stages the lateral walls have not yet formed. By stage 12 the connection with the yolk sac has narrowed such that the midgut has ventral walls cranially and caudally; this connection is reduced to a yolk stalk containing the vitello-intestinal duct during stage 13, at which time the yolk sac appears as a sphere in front of the embryo. Posterior to the midgut the splanchnopleuric coelomic epithelia converge forming the dorsal mesentery; ventrolaterally the intraembryonic coelom is in wide communication with the extraembryonic coelom. At stage 14 the midgut has increased in length more than the axial length of the embryonic body and, with elongation of the dorsal mesentery, it bulges ventrally, deviating from the median plane.

Umbilical Cord

The formation of the lateral body walls brings the zone of connection of ectoderm and amnion to

the ventral part of the body. The embryo undergoes a rotation within the chorion so that it now has its ventral surface opposite the placenta. Amnion now covers the connecting stalk with its umbilical vessels and the elongating yolk stalk, thus limiting the previously wide connection of the intra- and extraembryonic coeloms to a much narrower channel, an umbilical coelom, around the vitello-intestinal duct within the base of the forming umbilical cord.

The yolk stalk consists of:


- a central endodermal yolk duct
- a covering of splanchnopleuric extraembryonic mesenchyme (vitelline), which carries the vitelline arteries and veins
- a covering of splanchnopleuric coelomic epithelium.

The connecting stalk consists of:


- a central endodermal allanto-enteric duct (diverticulum)
- a covering of splanchnopleuric extraembryonic mesenchyme, conveying the umbilical (allantoic) vessels to and from the chorion
- a covering of splanchnopleuric coelomic epithelium.

Both the yolk stalk and the connecting stalk are enclosed by, from within and out:


- the somatopleuric coelomic epithelium
- the somatopleuric extraembryonic mesenchyme of the amnion
- the amniotic epithelial cells.

The mesenchymes of all three groups will fuse (see below), except in the embryonic base of the umbilical cord where it persists as the umbilical coelom. For more information on the umbilical cord see page 158 .

Primary Intestinal (or Midgut) Loop

This is present at stage 15 when a bulge, the *caecal bud*, can be discerned on the lower limb of the loop, caudal to the yolk stalk, which arises from the apex or summit of the loop (3.76 ). Later, the original proximal limb of the loop moves to the right and the distal limb to the left. Interestingly the longest portion of the dorsal mesentery is at the level of the yolk stalk; less relative lengthening occurs near the caudal end of the duodenum or the cranial half of the colon. The midgut extends into the umbilical coelom having already rotated through an angle of 90°, anticlockwise viewed from the ventral aspect. This relative position is roughly maintained so long as the protrusion persists, during which time the proximal limb which forms the small intestine elongates greatly, becoming coiled, and with its adjacent mesentery adopting a pleated

appearance. The origin of the root of the mesentery is initially both median and vertical while at its intestinal attachment it is elongated (like a ruffle) and folded along a horizontal zone. The mesenteric sheet has spiralled with its contained vessels through 90°. The distal, colic, part of the loop elongates less rapidly and has no tendency to become coiled. By the time the fetus has attained a length of 40 mm (10 weeks), the peritoneal cavity has enlarged and the relative size of the liver and mesonephros is much less. The re-entry of the gut occurs rapidly and in a particular sequence during which it continues the process of *rotation*. The proximal loop returns first, with the jejunum mainly on the left and the ileum mainly on the right of the subhepatic abdominal cavity. Both coils of jejunum and ileum, however, as they re-enter the abdominal cavity slide inwards over the right aspect of the descending mesocolon, thus displacing the descending colon to the left and the transverse colon passes superiorly to the origin of the root of the mesentery. The caecum is the last to re-enter and at first lies on coils of ileum on the right. Later development of the colon leads to its elongation and to establishment of the hepatic and splenic flexures.

During the period when the midgut loop protrudes into the umbilical coelom the edges of the ventral body wall are becoming relatively closer, forming a more discrete root for the umbilical cord. Somatic mesenchyme, which will form the ventral body wall musculature, migrates into the somatopleuric mesenchyme and passes ventrally toward the midline (see p. 270 ). When the midgut loop is abruptly returned to the abdominal cavity the more recognizable umbilical cord forms (see previously). The vitello-intestinal duct and vessels involute, the cranial end of the allantois becomes thinned and its lumen partially obliterated; it forms the urachus. The mesenchymal core of the umbilical cord is derived by coalescence from somatopleuric amniotic mesenchyme, splanchnopleuric vitello-intestinal (yolk sac) mesenchyme, and splanchnopleuric allantoic (connecting stalk) mesenchyme. These various layers become fused and are gradually transformed into the viscid, mucoid connective tissue (Wharton's jelly) which characterizes the more mature cord. The changes in the circulatory system result in a large cranially oriented left umbilical vein, the right umbilical vein regressing, and two spirally disposed umbilical arteries.

Mucosal Development

The exact timing of the cellular morphogenesis of the gut is difficult to establish, especially as the gut undergoes a proximodistal gradient in maturation; developmental differences between parts of the small intestine or colon have not yet been correlated with age. The endodermal cells of the small intestine proliferate forming a layer some three to four cells thick with mitotic figures throughout. From 7 weeks (according to some accounts) blunt projections of the endoderm have begun to form in the duodenum and proximal jejunum; these are the developing villi which increase in length until in the duodenum the lumen becomes difficult to discern. The concept of occlusion of the lumen and recanalization which is described in many accounts of development does not match with the cytodifferentiation occurring in the gut epithelia. By 9 weeks the duodenum, jejunum and proximal ileum have villi and the remaining distal portion of ileum develops villi by 11 weeks. The villi are covered by a simple epithelium. Primitive crypts, epithelial downgrowths into the mesenchyme between the villi, appear between 10 and 12 weeks similarly along a craniocaudal progression. The morphological appearance of the small intestine

is similar to the adult's by 16 weeks.

Whereas mitotic figures are initially seen throughout the endodermal layer of the small intestine prior to villus formation, by 10 to 12 weeks they are limited to the intervillous regions and the developing crypts. It is believed that the adult-like turnover of cells may exist when rounded-up cells can be observed at the villus tips, in position for exfoliation. The epithelial cells show an appropriate differentiation before 9 weeks when the absorptive enterocytes have microvilli at their apical borders. An apical tubular system appears at this time composed of deep invaginations of the apical plasma membrane and membrane-bound vesicles and tubules; there is also the appearance of many lysosomal elements (meconium corpuscles) in the apical cytoplasm. These latter features are more developed in the ileum than jejunum; they are most abundant at 16 weeks and diminish by 21 weeks. There are abundant deposits of glycogen in the fetal epithelial cells; it is suggested that prior to the appearance of hepatic glycogen the intestinal epithelium serves as a major glycogen store (Menard 1989📖). Goblet cells are present in small numbers by 8 weeks, Paneth cells differentiate at the base of the crypts in weeks 11 and 12 and enteroendocrine cells appear between weeks 9 and 11.

Meconium can be detected in the lumen of the intestine by the 16th week. It is derived from swallowed amniotic fluid which contains vernix and cellular debris, salivary, biliary, pancreatic and intestinal secretions and sloughed enterocytes. As the mixture passes along the gut, water and solutes are removed and cellular debris and proteins concentrated. Meconium contains enzymes from the pancreas and proximal intestine in higher concentrations in preterm than full-term babies (Koldovsky 1989📖).

Muscularis Layer of the Small Intestine


This is derived from the splanchnopleuric mesenchyme as in other parts of the gut. At 26–30 weeks the gut shows contractions without regular periodicity; from 30–33 weeks repetitive groups of regular contractions were seen in preterm neonates (Ruckebusch 1989📖).

Serosa of the Midgut




Possessing a dorsal mesentery alone, the movement of the root of this dorsal mesentery and the massive lengthening of its enteric border to match the longitudinal growth of the gut tube reflects the spiralizing of the midgut loop in the umbilical coelom. Specific regions of adherence of the serosa and parietal peritoneum result in the final disposition of parts of the small and large intestine in the peritoneal cavity. For an account of this see page 192📖.


Primitive Hindgut

Just as the foregut has an extensive, ventral endodermal diverticulum which contributes to a system separate from the gut, so too the hind-gut has a ventral diverticulum, the *allantois*, fated for a different system. However, unlike the respiratory diverticulum of the foregut the allantois is

formed very early in development, prior even to formation of the embryonic endoderm and tail folding. With the reorganization of the caudal region of the embryo at stage 10, part of the allantois is drawn into the body cavity. The early embryonic hindgut thus consists of a dorsal tubular region extending from the caudal intestinal portal to the cloacal membrane, and a ventral blind-ending allantois extending from the cloacal region into the connecting stalk. The slightly dilated cavity, lined by endoderm, that cranially receives the enteric hindgut proper and the root of the allanto-enteric diverticulum is termed the *endodermal cloaca*. It is closed ventrally by the cloacal membrane (endoderm opposed to proctodeal ectoderm); it also has, transiently, a small recess of endoderm in the root of the tail, the *postanal gut*. As elsewhere, the hindgut, allantois and endodermal cloaca are encased in splanchnopleuric mesenchyme. Proliferation of the mesenchyme and endoderm in the angle of junction of hindgut and allantois produces a *urorectal septum*. Continued proliferation of the urorectal septum and elongation of the endodermal structures thrusts the endodermal epithelium towards the cloacal membrane with which it fuses centrally, separating the presumptive rectum and upper anal canal (dorsally) from the presumptive urinary bladder and urogenital sinus (ventrally) (3.86 ). The cloacal membrane is thus divided into *anal* (dorsal) and *urogenital* (ventral) *membranes*. The nodal centre of division is the site of the future *perineal body*, the functional centre of the perineum.

Development of the Enteric Hindgut

The development of the large intestine, whether derived from mid- or hindgut seems to be similar. The proximal end of the colon can be first identified at stage 15 when an enlargement of a local portion of gut on the caudal limb of the midgut loop defines the developing *caecum*. An evagination of the distal portion of the caecum forms the *vermiform appendix* at stage 17. Apart from the embryonic studies of Streeter (1942  et seq) there is little information about the development of the large intestine in humans. A classic study by Johnson (1914  has not yet been superseded. The early endodermal lining of the colon appears stratified, with mitoses occurring throughout the layers. A series of longitudinal folds arise initially at the rectum and caecum and later in the regions of colon between these two points. The folds segment into villi with new villi forming between them while the developing mucosa invaginates into the underlying mesenchyme between the villi to form glands which increase in number by splitting longitudinally from the base upwards. The villous nature of the developing human colon has been confirmed (Bell & Williams 1982 ); the villi gradually diminish in size and are absent by the time of birth.

The similarity of development of the small and large intestines is further mirrored in the cytological differentiation. Fetal gut from 11 weeks shows dipeptidase activity in the colon as well as in the small intestine (Potter 1989 ). Throughout preterm development *meconium* corpuscles are seen in the colon as in the small intestine; they are believed to be the phagocytosed remains of neighbouring cells which have died as a result of programmed cell death.

There is little direct evidence of colonic function in the human fetus and neonate; however, the specific results of mammalian studies are being correlated to human studies where possible

(Potter 1989^[1]). A number of distinct and important differences between the function of adult and fetal colon have been reported. The absorption of glucose and amino acids does not take place through the colonic mucosa in adult life; however, there is evidence of direct absorption of these nutrients during development. At birth the normal cycle of bile acids is not mature. In the adult, bile is secreted by the liver, stored in the gallbladder then secreted into the intestine where it is absorbed by the jejunum and ileum. In the fetus and neonate, the transport of bile acids from the ileum by an active process does not occur, allowing bile salts to pass on into the colon. In the adult the presence of bile salts in the colon stimulates the secretion of water and electrolytes resulting in diarrhoeal syndrome; the fetal and neonatal colon however seems protected from this effect. The colon is not considered a site of significant nutrient absorption in the adult; however, neonates are unable to assimilate the full lactose load of a normal breast feed from the small intestine and a large proportion of it may be absorbed from the colon. Thus it is suggested that the colon fulfils a slightly different role in the preterm and neonatal period, conserving nutrient absorption and minimizing fluid loss until the neonate has adjusted to extrauterine life, oral feeding and the establishment of the symbiotic bacterial flora.

Muscularis Layer of the Colon

This is present and functioning by the 8th week, when peristaltic waves have been observed. The specific orientation of the longitudinal muscle layer into *taeniae coli* occurs in the 11th to 12th weeks when haustra appear. The enteric nerves are present in Meissner's and Auerbach's plexuses at 8 and 12 weeks respectively, with a craniocaudal migration of the nerves. A normal distribution of ganglion cells has been noted in preterm babies of 24 weeks, although there is a region devoid of ganglia just above the anal valves (Grand et al 1976^[2]). The puborectalis muscle appears in 20–30-mm embryos, following opening of the anal membrane.

Mesenchymal proliferation occurs around the rim of the ectodermal aspect of the anal membrane which thus comes to lie at the bottom of a depression, the *proctodeum*. With the absorption and disappearance of the anal membrane the anorectum communicates with the exterior. The lower part of the anal canal is formed from the proctodeal ectoderm and underlying mesenchyme, but its upper part is lined by endoderm. The line of union corresponds with the edges of the anal valves in the adult (p. 1780^[3]). In the fourth and fifth weeks a small part of the hindgut, the postanal gut, projects caudally beyond the anal membrane (towards the region of the tail); it usually disappears before the end of the fifth week. The dual origin of the anal canal is reflected by differences in arterial supply, venous and lymphatic drainage, innervation and epithelial specialization (summarized on p. 1782^[4]).

Lymphoid Tissue in the Developing Gut

The neonatal gut becomes colonized by a range of bacterial flora, some of which exists in a symbiotic relationship with its host, some of which may be considered pathogenic. The gut therefore has a significant function in the defence of the body which can be seen in the development of the lymphoid tissues of the gut. Individual *lymphocytes* appear in the lamina propria of the gut from about week 12 of development and lymphoid aggregates, *Peyer's patches*, have been noted between 15 and 20 weeks; it is not clear whether these cells migrate in

from distant sources or differentiate from the investing mesenchyme. The endodermal epithelium overlying the lymphoid aggregates is often distorted into a dome shape; the cells are a mixed population of enterocytes, endocrine cells, a reduced population of goblet cells and unique absorptive cells termed *M cells* (*membrane or microfold*). These latter cells are specialized to provide a mechanism for the transport of micro-organisms and intact macromolecules across the epithelium to the intraepithelial space and lamina propria where the underlying macrophages and lymphocytes are present. M cells have been observed in the fetus by 17 weeks (Moxey & Trier 1978^[1]); it is believed that they are formed by a specialized epithelial/mesenchymal interaction of the endoderm and underlying lymphoid type mesenchyme.

There are similarly specialized epithelial cells *between* the enterocytes. *Intraepithelial leucocytes* account for some 15% of the epithelial cells of the gut in the adult. They have been observed at 11 weeks' development, with a distribution of approximately three intraepithelial leucocytes per 100 gut epithelial cells (Orlic & Lev 1977^[2]). They are thought to be T and B lymphocytes. For an account of the very complex development of the immune cells of the gut consult Butzner and Befus (1989^[3]).

Innervation of the Gut

The gut is innervated by the *enteric nervous system (ENS)* which unlike other components of the PNS, e.g. the automatic nervous system, can mediate reflex activity independently of control by the brain and spinal cord (see p. 235^[4]). The number of enteric neurons is very large, in the same order of magnitude as the number of neurons in the spinal cord (Furness & Costa 1980^[5]). The source of enteric neurons is from somite levels 1–7 and from 28 onwards. Crest cells invade the gut after migrating ventrally and via the dorsal mesentery. The glia cells associated with the gut have been identified as arising from similar levels; these cells are unlike Schwann cells and more closely resemble astrocytes. The precursors of enteric neurons colonize the bowel fairly rapidly in mammals (Gershon 1987^[6]); see also page 234^[7].

Development of the Allantoic Hindgut


After proliferation and migration of the *urorectal septum*, the cloacal region is divided into a dorsal portion, the putative rectum, and a ventral portion which can be subdivided into three regions:

- a cranial vesico-urethral canal, continuous above with the allantoic duct
- a middle, narrow channel, the pelvic portion
- a caudal, deep, phallic section, closed externally by the urogenital membrane (2.122^[8]).

The second and third parts together constitute the *urogenital sinus*. The paired mesonephric ducts, part of the urogenital system, fuse with the posterior wall of the urogenital sinus and

become partially absorbed into its wall. They are incorporated over a triangular region with the developing ureters (which arise from the mesonephric ducts) at the two upper angles of the triangle, and the mesonephric ducts at the lower apex. The mesonephric ducts arise from coelomic epithelium derived from the intermediate mesenchyme; this mesonephric epithelium thus contributes to the trigone of the bladder and dorsal wall of the proximal (superior) half of the prostatic urethra, i.e. as far as the opening of the prostatic utricle and ejaculatory ducts (or its female homologue the whole female urethral dorsal wall). The remainder of the vesico-urethral part forms the body of the bladder and urethra; its apex is prolonged to the umbilicus as a narrow canal of variable length in the fibrous urachus.

Urethra


Although an endodermally derived structure, which is involved in a series of epithelial/mesenchymal interactions both with the local mesenchyme and with the ectoderm (in much the same way as the pharyngeal arches and facial primordia), the urethra is usually dealt with within the urogenital system. The same applies for the *prostate gland* and *vagina* (both outgrowths of the lower urogenital sinus or urethra), and the other smaller glandular structures developing around the body orifices. For details of these organs see pages 213–214 .

It is worth noting at this stage that an interface between the endoderm and ectoderm occurs not only in the buccal region, but also at the proctodeum and the urethra. Specialized ectoderm/mesenchyme interactions produce folds and ridges that surround the ectoderm/endoderm junctions, which normally break down in utero. Generally epithelium which can be touched easily and has a somatic innervation is derived from ectoderm. In the buccal cavity and pharynx the ectoderm/endoderm zone is towards the posterior third of the tongue; touch here usually elicits the gag reflex, a protective response. In the anal canal the outer portion, distal to the anal valves, is ectodermally derived and somatically innervated; proximal to the valves the epithelium is endodermally derived and autonomically innervated. The urethra in the male is contained in the shaft of the penis with only a short invagination of ectoderm at the distal tip into the glans. In the female however the region of the early urethra remains open to form the vestibule into which the definitive urethra and vagina open. It is believed that these regions are invaded by ectoderm; they are innervated by somatic nerves.

Anomalies of the Gut

Duodenal atresia is the most common small bowel obstruction. It occurs commonly because of the presence of a membrane across the lumen although 20% of cases are associated with annular pancreas. The diagnosis of this condition relies on the demonstration of the 'double-bubble sign' on ultrasound, due to the simultaneous distension of the stomach and the first portion of the duodenum.

Bowel atresia usually is not due to a problem with organogenesis but more likely due to a vascular insult during fetal life.

If the midgut loop fails to return to the abdominal cavity at the appropriate time a range of ventral defects can result ranging from minor to major. An *umbilical hernia* occurs when loops of gut protrude into a widened umbilical cord at term. The degree of protuberance may increase if the infant cries, raising the intra-abdominal pressure; however, these herniae resolve usually without treatment. *Omphalocele* is a ventral wall defect with midline herniation of the intra-abdominal contents into the base of the umbilical cord. Herniated viscera are covered by the peritoneum internally and amnion externally. Omphalocele range in size from a large umbilical hernia to a very large mass containing most of the visceral organs. *Gastroschisis* is a para-umbilical defect of the anterior abdominal wall associated with evisceration of the abdominal organs. The organs are not enclosed in membranes. Gastroschisis is thought to result from peri-umbilical ischaemia caused by vascular compromise of either the umbilical vein or arteries. Gastroschisis can be detected by prenatal ultrasonography (see p. 341 .


Bladder and cloacal exstrophies are considered with development of the urogenital systems.

Imperforate anus is a term used to describe many different anorectal malformations. Minor anomalies are easily treated with an excellent prognosis; however, other defects are often associated with important anatomical deficiencies. The principal concern in all cases is the degree of bowel control, urinary control and in some cases sexual function which is compromised by the condition.

Development of the Body Cavities

Peritoneum and Omental Bursa

Students addressing themselves de novo to topographical descriptions of the mature peritoneal cavity with its complex parietes, 'sessile' (or retroperitoneal) organs, peritonealized organs with their mesenteries (termed ligaments, folds or omenta in different locations), mesenteric contents and lines of reflexion and recesses, fossae, 'gutters', spaces and bursae, face a formidable task. Comprehension is often lacking, and much of what is merely rote retention quickly recedes. Preliminary study of a concise account of the development of an organ and its surroundings, or a survey of the whole region, is valuable; thus frequent cross reference should be made between any field of study of a mature organ, and its ontogeny.

The emergence of an embryonic disc and proliferation and spreading of intraembryonic mesoblast in the progressively pear-shaped, bilaterally symmetric, trilaminar embryo has been described (p. 142 ). The initial appearance of a median, rostrocaudally disposed protocardiac area, buccopharyngeal membrane, notochord, Hensen's node and primitive streak, is accompanied throughout the length of the notochord by laterally placed paraxial mesenchyme and lateral plate mesenchyme (the intermediate mesenchyme develops somewhat later). The embryonic plate is, at the disc margins, ringed by a band of extraembryonic mesoblast where somatopleure (amnion) and splanchnopleure are confluent; with growth, the band meets and

fuses with the spreading lateral plate. The fusion of these three populations of mesoblast occurs at the *junctional zone*; the latter completely encircles the early embryonic plate periphery, but beyond the cranial tip of the notochord involves rostral streams of mesoblast from the primitive streak which skirt the buccopharyngeal membrane, meet in the protocardiac area and continue into a substantial part of the junctional zone. Caudally, mesoblast streams from the primitive streak, skirts the cloacal membrane and blends with the connecting stalk around its contained allantoic duct (p. 144📖). Median cavitation in the protocardiac area and its confluence with multiple clefts in each lateral plate forms a horseshoe-shaped *intraembryonic coelom* which is bounded dorsally by somatopleuric coelomic epithelium, ventrally by splanchnopleuric coelomic epithelium, medially by paraxial (later, intermediate) mesenchyme and, initially, rostrolaterally by the junctional zone (see below). Loss of the junctional zone in the caudal half of the embryonic margins establishes communication between intraembryonic and extraembryonic coeloms. Head, tail and lateral folding imposes craniocaudal reversal of the headward and tailward structures, assumption of embryonic form, circumscription of an umbilicus, enfoldment of a splanchnopleuric gut tube and definition of the primary body cavities and coelomic regions. The manner of their separation into definitive pericardial, pleural and peritoneal cavities has been described (see p. 180📖); particular emphasis is placed on the invasion and splitting of the somatopleure with expansion of the secondary pleural cavities, and submergence of the pericardium. The positional changes and multiple derivations of the diaphragm are discussed in musculoskeletal development (see p. 270📖). The intraembryonic encompassing of a splanchnopleuric gut tube, its allocation into foregut, midgut and hindgut regions and a summary of their derivatives have been considered (see above). Also the period of extrusion of the midgut loop into the umbilical exocoelom, concomitant differential growth and varying degrees of rotation of almost all parts of the subdiaphragmatic gut, return of the midgut loop, and profound serous membrane modifications are traced (pp. 190📖 et seq.).

Some repetition is unavoidable but, where apposite, brief summaries are appended to circumvent unacceptable degrees of cross-referencing; in other locations, further details and systematic names are given, when alternative morphogenetic events occur, but are infrequently mentioned in introductory accounts. Only those viscera developed in direct apposition to one of the primary coelomic regions, or a secondary extension of the latter, retain a partial or almost complete visceral serous cover. No coelomic cavitation occurs in the rostral and caudal ends of the embryo, i.e. the cranium, cervical vertebrae, buccal cavity, pharynx and their mural derivatives; similarly, the lower third of the rectum, anal canal, coextensive vagina and lower urinary tract. In all these cases, derived structures are embedded in unsplit *mixed somatovisceral mesenchyme*, often with accessions from the neural crest. (The possible classification of certain groups of **both** craniocervical **and** caudoperineal muscles and their nerves as 'special visceral' should be reviewed.) The cervicothoracic oesophagus is encased in prevertebral, retrotracheal and retrocardiac mesenchyme and develops no true dorsal or ventral mesentery. In the lower thorax the oesophagus inclines ventrally anterior to the descending thoracic aorta; the dorsocaudally sloping midline diaphragm between oesophageal and aortic orifices may be homologized with part of a *dorsal meso-oesophagus*; in the same manner, a ventral midline diaphragmatic strip may be considered a derivative of a *ventral meso-oesophagus*. At superior and intermediate thoracic levels parts of the lateral aspects of the oesophagus come into closer relation to the secondary, mediastinal, *parietal* pleura (p. 181📖).

The alimentary tube from the diaphragm to the commencement of the rectum possesses, throughout its length, initially, a sagittal dorsal mesentery; its line of continuity with the dorsal parietal peritoneum (i.e. its 'root' or 'line of reflexion') is, evidently, also midline. The abdominal foregut, from the diaphragm to the future hepatopancreatic duodenal papilla, also has a ventral mesentery. The latter extends from the ventrolateral margins of the abdominal oesophagus and, as yet 'unrotated', primitive stomach and proximal duodenum, cranially to the pars diaphragmatica of the septum transversum, anteriorly to the ventral abdominal wall to the level of the cranial rim of the umbilicus; caudally (between umbilicus and duodenum) it presents a crescentic free border. The midgut and hindgut have no ventral mesentery; thus the pleural and supra-umbilical peritoneal cavities are initially (and transiently) bilaterally symmetrical above the umbilicus; below, the peritoneal cavity is freely continuous across the midline ventral to the gut.

A few general developmental points may be mentioned. Some organs, for example the lung, despite the extensive pulmonary growth and wide cavitation of a secondary pleural cavity, retain a relatively simple visceroparietal serous membrane disposition. The lung root, with its bronchial, neurovascular and lymphatic radicles is circumscribed by a comma-shaped line of reflexion, the dependent 'tail' or pulmonary ligament accommodating calibre variations in the contained tubes. Other organs with a single dorsal mesentery may undergo changes varying from slight to profound. Thus mesenteric changes are an integral accompaniment of alterations in their visceral contents including differential, sometimes asymmetric growth, regional or progressive degeneration, repositioning such as sequential extrusion, retrusion, rotation, spiralization and relative ascent or descent. The *parietovisceral line of reflexion* of the originally midline dorsal mesentery may become oblique (see the jejuno-ileal mesentery) or transverse (see transverse mesocolon), angular (see sigmoid mesocolon) or complex and highly curved (see dorsal mesogastrium). In other locations, the events are more pronounced, the dorsal mesentery is lost entirely and the organ becomes 'sessile' or 'retroperitoneal', connective tissue only separating its external (usually posterior) surface from the abdominal parietes or the surface of another organ; the peritoneum is limited to some or all of its (usually) ventral and perhaps ventromedial and also ventrolateral surfaces. The resulting parietovisceral or intervisceral depressions or grooves are called pouches, fossae or gutters in different locations. The term *recess* has two distinct connotations, *embryological* (see below), and in *mature topography* where it denotes a peritonealized, blind-ended channel, extending from a major region of the peritoneal cavity, continuing in a retrovisceral or paravisceral position, and often partly enclosed by one or two vascularized (or avascular) peritoneal folds.





The movements of lines of reflexion, assumption of a retroperitoneal site and other changes, are often held to affect relatively large endodermal 'organs' encased in a **fine** layer of splanchnic mesenchyme which is continued to the parietes by an equally tenuous, but vascularized, mesentery. With growth and also shape and positional changes, part or the whole of the mesentery lies against the parietal peritoneum; their apposed surfaces fuse and are absorbed. Thus the line of reflexion is altered, or the organ becomes retroperitoneal; further, mesenteric neurovascular bundles lie ventral to structures derived primarily from the intermediate mesenchyme. Such mechanisms are significant throughout the subdiaphragmatic gut, but are predominant in the small and large intestine. However, such views fail to recognize the ability of

all serous membranes to vary their thickness, lines of reflexion, disposition, 'space' enclosed and their channels of communication, by areal and thickness growth on one aspect combined with cavitation leading to expanding embryonic recess formation on the other. The ventral and dorsal foregut mesenteries are relatively large (composed of mesenchyme sandwiched between two layers of splanchnopleuric coelomic epithelium), compared with the slender endodermal tubes they encase. A complex series of recesses develop in the splanchnopleuric mesenchyme, become confluent, and with foregut rotation, differential growth of stomach, liver, pancreas and spleen, and completion of the diaphragm, the territories of the greater sac and lesser sac (omental bursa) are delimited, and the mesenteric complexes of these organs (omenta and 'ligaments') are defined (see below).

It is convenient to first consider the mesenteries of the small and large intestine after rotation and the principal growth patterns have been achieved and the developing pancreas is becoming retroperitoneal. Most of the duodenal loop encircles the head of the pancreas and is retroperitoneal, the peritoneum covering principally its ventral and convex aspects. Exceptions are a short initial segment of the superior (first) part; this is more completely peritonealized having the attachments of the right margins of the greater and lesser omenta; peritoneum is lacking when there is close apposition of the transverse colon to the descending (second) part, or where the latter is crossed by the root of the transverse mesocolon; also where *the* mesentery crosses the transverse (third) part, and descends across the ascending (fourth) part from its upper extremity at the duodenojejunal flexure. In addition to the main peritoneal relations of the duodenum just mentioned, one or more of up to six different *duodenal recesses* may develop. Their variations in shape and size, their intestinal, mesenteric and vascular relations and, when adequately recorded, their frequencies and disposition of their orifices are given on page 1744 [\[1\]](#), and will not be repeated here.

The succeeding small intestine (jejunum and ileum) from the duodenojejunal flexure to the ileocaecal junction ('valve') undergoes, from a mesenteric standpoint, less modification of its embryonic form than other gut regions. Its early dorsal mesentery (no ventral component is present here, or caudally) is a continuous, single (but structurally bilaminar) sheet, with its parietal attachment—line of reflexion, or 'root'—in the midline. Usually, with development and the mechanisms mentioned above, the attachment of the root becomes an **oblique** narrow band from the left aspect of the second lumbar vertebra to the cranial aspect of the right sacro-iliac joint. Thus, from above downwards, it crosses the ascending and transverse parts of the duodenum, abdominal aorta, inferior vena cava, right psoas major and many structures related to these. For dimensions, contents and other details see pages 1765 [\[1\]](#), 1772 [\[1\]](#). Formal names—mesojejunum, meso-ileum—are seldom used; *the mesentery* is universal.

The caecum and vermiform appendix, as stated, arise as a diverticulum from the *antimesenteric* border of the caudal limb of the midgut loop and thus the caecum possesses no primitive mesocaecum. These regions of the gut undergo long periods of growth, often asymmetrical, and their final positions, dimensions and general topography show much variation (pp. 1774 [\[1\]](#), 1775 [\[1\]](#)). The caecum is usually quite mobile with its lateral, medial, ventral, caudal and most of its dorsal surfaces clothed with visceral peritoneum. Peritoneum may be lacking dorsally near its continuation into the ascending colon. This arrangement is, however, usually complicated by the

presence of two or three *caecal recesses*, bounded on one or more aspects by *local peritoneal folds* some of which carry vascular pedicles. For further details of the recesses (*superior* and *inferior ileocaecal* and *retrocaecal*) and folds (*lateral* and *medial parietocaecal*, *vascular caecal* and *ileocaecal*) see page 1744. The vermiform appendix is also almost wholly clothed with visceral peritoneum, derived from the diverging layers of its rather diminutive *mesoappendix*. The latter, of triangular profile, carrying the appendicular vessels, lymphatics and nerves, is further detailed on page 1743; it appears as a continuation of 'the' mesentery near its ileocaecal junction. Despite the opening remarks above, therefore, the mesoappendix should perhaps be regarded as a direct derivative of the primitive dorsal mesentery; on this view, a similar status for the vascular fold of the caecum should be considered. With approaching completion of differential growth, rotation and circumabdominal displacement of the colonic gut, until the fourth month, this part of the gut retains its primitive dorsal mesentery (mesocolon) throughout. Its original root is still vertical in the dorsal midline, from which it diverges widely, roughly as an incomplete, flattened pyramid, to reach its colonic border (at the future taenia mesocolica). During the fourth and fifth months substantial areas of the primitive mesocolon adhere to, then fuse with, the parietal peritoneum; thus some colonic segments become sessile while others have a shorter mesocolon with an (often profoundly) altered parietal line of attachment (root). In one series of 100 specimens studied (Treves 1885) the most common arrangement occurred in about 50%; in these a transverse and a sigmoid mesocolon persisted and the sessile state affected the ascending colon, right (hepatic) flexure and the descending colon. In the remainder, either the ascending or descending, or both, colonic segments also retained a mesocolon (varying from a localized 'fold' to a complete mesocolon). When sessile, the ventral, medial and lateral aspects of the ascending or descending colon are clothed with peritoneum, the protrusion of the viscus producing medial and lateral peritoneal *paracolic gutters* on each side. The multiple topographical relationships of these parts of the colon and particularly the dorsal structures, with a mere fibro-areolar separation, are detailed on page 1745. This form of apposition to underlying structures proceeds from the ascending colon to include the right colic (hepatic) flexure, and thence continues antero-inferiorly to the left, thus involving the right-sided initial segment of the transverse colon. The right flexure is in contact with the caudal part of the ventrolateral surface of the right kidney, the colonic peritoneum passes cranially clothing this part of the kidney; sometimes reaching the rim of the right suprarenal. In either event, the peritoneum is next reflected ventrally as the lower layer of the coronary ligament to the dorsum of the right lobe of the liver: this renal peritoneum forms the floor of the *hepatorenal pouch* (of Morison). The pouch, in addition to its topographical relations with colon, kidney, suprarenal and liver, has clinically significant associations with the right lateral paracolic gutter, the epiploic foramen—its boundaries and their many contents—the vestibule of the mature omental bursa, the gallbladder and biliary ducts. (Numerous references are made to all the foregoing in Section 8). The right extremity of the transverse colon, as mentioned, is also sessile, fibro-areolar tissue separating it from the anterior aspect of the descending (second) part of the duodenum and the corresponding aspect of most of the head of the pancreas. The remainder of the transverse colon, up to and including the left (splenic) colic flexure, is almost completely peritonealized by the diverging layers of the *transverse mesocolon*. The root of the latter reaches the neck and whole extent of the anterior border of the body of the pancreas. The long axis of the definitive pancreas lies **obliquely**; also, the splenic colonic flexure is considerably more rostral than the hepatic flexure; in accord, the root of the mesocolon curves obliquely upwards as it crosses the upper abdomen from right to left. As it expands, the postero-inferior wall of the greater omental

part of the bursa omentalis (see below) gradually covers, becoming closely applied to, the transverse mesocolon and its contained colon, finally projecting beyond the latter. Craniocaudal adherence now occurs between the omental wall and the pericolonic and mesocolonic layers. In the mature condition, therefore, a cursory examination suggests that the transverse colon is connected to the posterior abdominal structures by a single mesenteric sheet; close inspection, however, shows this to be a compound structure, quadrilaminar in nature (p. 1743^[1]).

The left colic flexure receives much of its peritoneal covering from the left extremity of the transverse mesocolon; it is also often connected to the parietal peritoneum of the diaphragm over the tenth and eleventh ribs by a *phrenicocolic ligament*. The latter sometimes blends with a *presplenic fold* that radiates from the gastrosplenic ligament. The descending colon becomes sessile; it commences over the inferolateral border of the left kidney and first passes almost vertically in the depression between psoas major, quadratus lumborum and the aponeurotic origin of transversus abdominis, to the level of the iliac crest; here it inclines inferomedially crossing iliacus and psoas major to reach the brim of the true pelvis where it continues as the sigmoid colon. The numerous structures intervening between the posterior aspect of the descending colon and its fibromuscular 'bed' are detailed on page 1777^[2]. Contrary to often stated (but unsupported) assumptions, it has now been clearly demonstrated (Kanagasuntheram 1957^[3]) that the process of fusion and obliteration of both ascending and descending mesocolons commences laterally and progresses medially.

The sigmoid colon is ultimately most variable in its length and disposition, with numerous topographical relationships (p. 1777^[2]). It retains its dorsal mesocolon, but the initial midline dorsal attachment of its root is considerably modified in its definitive state. The latter is commonly described as an inverted V which is, however, asymmetric, and, other than the rectal termination of its right limb, lies wholly to the left of the midline. The apex of the V lies ventral to the bifurcation of the left common iliac artery; this separates it from the cranial part of the left sacro-iliac joint. The left limb of the V is shorter than the right and, bearing the inferior left colic (sigmoidal) vessels, is almost horizontal, inclining only slightly cranially as it is traced lateromedially from its origin to the apex. The right limb carries the superior rectal vessels; it is longer than the left and about 45° to the vertical, as it extends from the apex to its midline termination (at the cranial end of the rectum) on the ventral aspect of the third sacral vertebra. For details of the relationships and contents of the sigmoidal mesocolon, also the presence, form and age dependency of its associated *intersigmoid apical recess*, see pages 1743^[1], 1744^[2], 1778^[2].



The rectum continues from the ventral aspect of the third sacral vertebra to its anorectal (perineal) flexure antero-inferior to the tip of the coccyx, the distance, of course, changing with age. All aspects are encased by mesenchyme; the early dorsally placed mass is named, by some authorities, the *dorsal mesorectum*. The latter does not form a true mesentery, however, but with progressive skeletal development it reduces to a woven fibroareolar sheet with patterned variations in thickness and fibre orientation. The sheet is closely applied to the ventral concavity of the sacrum and coccyx, with numerous fibromuscular and neurovascular elements enclosed (p. 1779^[2]). Thus, the rectum becomes sessile, visceral peritoneum being restricted to its lateral and ventral surfaces. With the disappearance of the postanal gut by the end of the fifth week, the

ventrolateral peritoneum reaches the superior surface of the pelvic floor musculature, and this persists until late in the fourth month. In this period the ventral rectal peritoneum of the male is reflected to cover the posterior surface of the prostate and bladder trigone and associated structures; the female initially receives a reflexion covering almost the whole posterior aspect of the vagina, thence continuing over the uterus. Subsequently, the closely apposed walls of these deep peritoneal pouches fuse over much of their caudal extent, their mesothelia are lost, and the organs have an intervening, bilaminar (surgical separable), fibrous stratum. The latter is the masculine rectovesical fascia and posterior wall of the prostatic sheath; its female homologue is the posterior part of the fibrous envelope of the vagina, that intervenes between its middle two-fourths and the rectum. Thus the proximal third of the rectum has a peritoneal tunic ventrolaterally; the lateral extensions are triangular—deep proximally and tapering to an acute angle when the middle third of the rectum is reached. Thereafter, the middle third has peritoneum restricted to its ventral surface where it forms the posterior wall of the shallower rectovesical or rectovagino-uterine pouch. The remaining rectum and anal canal are subperitoneal.




The many additional peritoneal eminences, ridges, folds, grooves, fossae and pouches that on varying time scales characterize the true pelvis and lower abdomen simply reflect the growth patterns of the subjacent viscera, vessels, some nerves or features of the parietes. They are mentioned either in the following pages, or with the appropriate organ in other sections of this text.

Subdiaphragmatic Foregut Peritoneum

Subdiaphragmatic foregut peritoneum is complex in its definitive topography and, whilst having relatively simple symmetrical origins, becomes progressively more complicated during development. The latter involves rapidly changing three-dimensional arrays of serosal visceral surfaces, mesenchymal masses and mesenteric sheets. Difficulties are compounded by the (rather surprising) paucity of structural ontogenetic accounts, the obligatory absence of human experimental data, some slackening of surgical interest in topographical minutiae and the adoption of simplified basic instructional courses. Here a few paragraphs offer scant justice to the topic and space limitations permit only brief allusion to the (often 'investigator specific') variations in terminology commonly encountered. Classic investigations, reviews and bibliographies are found in Broman (1904^[1], 1938^[2]); these may be contrasted with Kanagasuntheram (1957^[3]).

Some features have already been mentioned in other contexts, but will be summarized here to allow addition, deletion, criticism and suggested alternatives to the nomenclature and causal mechanisms invoked. (Throughout these and previous sections, reference should be made to **3.76A-H**  and **3.79A-E** .



The subdiaphragmatic foregut includes, sequentially, the presumptive short terminal oesophagus, stomach and proximal duodenum (as far as, and including, the hepatopancreatic anlage). These subregions of the foregut initially (3–4 mm CR length) constitute a continuous endodermal tube

of roughly uniform calibre (but slightly flattened laterally in the gastric region), encased in a thick stratum of splanchnopleuric mesenchyme. The latter is linked from both its dorsal and ventral aspects with substantial blocks of splanchnopleuric mesenchyme that blend, respectively, with a wide dorsal midline strip of the body wall and diaphragm and the ventrolateral body wall and diaphragm, including the caudal base of the pericardium. Thus, at this early stage, the abdominal foregut with its associated mesenchymal masses comprises a thick sagittal septum, dividing the peritoneal coelom into right and left symmetric halves down to the level of the umbilicus. Until closure (p. 180 ) , each half communicates with a pleural coelom via a small pleuroperitoneal canal. With the appearance of recognizable subregions of the foregut, and of the liver, pancreas and spleen, and the hepatopancreatic duct systems, each undergoes asymmetric differential growth (sometimes interpreted as physical 'rotation' of the whole organ—see below), expansion and relative displacement. Concurrently, the splanchnopleuric mesenchyme that encases each organ forms its muscularis and connective tissue framework. The outer splanchnopleuric coelomic epithelium, where present, forms its visceral peritoneal surfaces (serosa). The mesenchyme between organs (e.g. between stomach and liver, stomach and pancreas, stomach and spleen, spleen and left kidney) and that extending from a viscus to the parietes, increases in area and becomes relatively thinner, while its visceroparietal lines of attachment are altered, as are, necessarily, their topographical planes. These events follow the development of *multiple small clefts* within the thick mesenchyme; their coalescence and secondary opening into the primitive peritoneal cavity rapidly follows and then further expansion of the *developmental recess* so formed. (The sequence of events closely mimics the formation of the primary intraembryonic coelom in the protocardiac area and lateral plate mesenchyme (see p. 148 )). It is **not**, as commonly described, a simple depression followed by excavation into one of the thick mesenchymal strata from the primary peritoneal cavity. These morphogenetic changes result in a marked asymmetry of the upper abdominal viscera, their mesenteries and associated subregions of the peritoneal cavity. The latter, although increasingly complex topographically, remains a single cavity with numerous intercommunicating regions, pouches and recesses. (The only small peritoneal sacs to separate completely from the main cavity are the *infracardiac bursa*—see below and p. 1751 —and the *tunica vaginalis testis*.)

Human Definitive Peritoneum

The human definitive peritoneal cavity (there are extreme species specializations), is commonly described as comprising a *lesser sac* and a *greater sac* of peritoneum.

Lesser Sac (or Definitive Bursa Omentalis)

This is compounded of a series of confluent recesses, mainly situated in the left, superior quadrant of the abdomen; however, it extends beyond the boundaries of the quadrant, crossing the median plane about 4 cm to the right and to a quite variable extent ventro-inferiorly. Briefly, it is intimately related to the retroperitoneal organs of the 'stomach bed', the transverse colon and its mesocolon, the postero-inferior surface of the stomach and mesenteries (omenta and ligaments) attached to its greater and lesser curvatures, the spleen and its ligaments, the caudate lobe and process of the liver and the diaphragm. (See below and p. 183  for details, also p. 197  for a discussion of the use of terms 'ligament' and 'omentum' in relation to peritoneal

features.) A little above the centre of its right margin, the cavity of the lesser sac connects with that of the greater sac, i.e. the whole remainder of the peritoneal cavity, through a vertical slit, with apposed but easily separable walls, the epiploic foramen (p. 1738📖).

Greater Sac

For practical convenience, this is often allocated into a number of subregions, or 'spaces': all, except two, are peritonealized, and all connect with neighbouring spaces, either by a wide direct or a more circuitous route. (In an overall peritoneal classification, as here, the cavity of the lesser sac and two extraperitoneal spaces are included.) The abdominal peritoneal cavity is (incompletely) divided by the transverse colon and its mesocolon into a *supracolic space* containing the foregut, its derivatives and mesenteries, and an *infracolic space* containing the midgut and hindgut with derivatives and mesenteries, already described. The supracolic space is subdivided into six: right and left *subphrenic*, *subhepatic* and *extraperitoneal* spaces. (The left subhepatic space is a rather inappropriate name for the omental bursa; the right extraperitoneal space is the 'bare area' of the liver; the left extraperitoneal space surrounds the left suprarenal and upper pole of left kidney.) The infracolic space is divided by the mesentery of the small intestine into **right** and **left** moieties, while external to the ascending and descending colon lie the *right* and *left lateral paracolic gutters*. All the latter are more or less directly continuous with the fossae and pouches of the lesser pelvis. (For further details of these various spaces see p. 1745📖 and throughout the account of the peritoneum in Gastrointestinal system.)

Abdominal Foregut

Abdominal foregut and its associated splanchnopleuric mesenchyme has an ontogenetic history too complex to receive more than a brief summary here; also the origin, admixture and final fate of some masses remain uncertain in the absence of experimental data. However, the nomenclature employed is, in some cases, a misleading simplification merely handed on in successive accounts over many decades. Sometimes, unproven mechanisms are held to operate which, on close examination, could not result in the well-known definitive topography. Some additional terms and divergences from current morphogenetic accounts are included.

Early embryonic nomenclature is often confined to the mesenchymal masses lying dorsal and ventral to the foregut. However, the thick mesenchymes surrounding the wall of the gut itself are equally prominent morphogenetically. Although continuous with, they cannot be allocated quantitatively as parts of, the dorsal and ventral masses. It is useful, therefore, to refer to them simply as *oesophageal*, *gastric* and *duodenal splanchnopleuric mesenchymes*. Some of the latter areas are **compound** in the sense that by cavitation and cleavage they may provide part of their thickness as *secondary extensions* of surrounding primary mesenteries with new lines of visceral attachment and areal expansion. These processes also result in modification of parietal lines of reflexion and related peritoneal cavity.

Septum Transversum

Septum transversum is the somewhat inappropriate name (but deeply ingrained in embryological literature) denoting all the mesenchyme that extends from the ventral aspect of the caudal foregut to the ventrolateral aspects of the supra-umbilical abdominal and lower thoracic walls; its rostral surfaces reach, and contribute to the caudal 'bases' of the pericardium and secondary pleural cavities; its caudal free surface arches from the duodenum to the cranial rim of the umbilicus. Dorsally it borders the pleuroperitoneal canals, is continuous with the foregut mesenchymes mentioned above, and contributes to the *caaval fold* (see below). Some of these features have been encountered elsewhere (e.g. pp. 152📖, 180📖) and will only receive a brief summary here. After head fold formation, the most rostral part of the septum transversum has the common sinuatrial chamber of the heart embedded in it, each sinuatrial horn of which receives the transeptal terminals of a vitelline, umbilical and common cardinal vein (for their fate see p. 180📖). As the pericardial cavity expands and the sinuatrial chamber 'rises' into it, septal mesenchyme follows (*pars pericardialis*) and condenses as the fibroserous diaphragmatic aspect of the pericardium. The intermediate stratum of septal mesenchyme (*pars diaphragmatica*) makes substantial contributions to the framework of the sternocostal parts of the diaphragm (3.79👁️). Additional extensions occur into the mesenchymatous bed in which the subhepatic–hepatic–hepatocardiac parts of the inferior vena cava develop (p. 324📖) and in relation to the expanding liver. Thus the *pars diaphragmatica* alone truly merits the name septum transversum and even this is both incomplete (until closure of the pleuroperitoneal canals) and also, with expansion of the lungs, secondary pleural cavities, liver and stomach, soon becomes less 'transverse' as it develops a marked convexity towards the thorax. Strong fibrous continuity between the perinodal area of the central tendon of the diaphragm and the overlying fibrous pericardium persists. Midline strips of diaphragm ventral and dorsal to the oesophageal hiatus are held by some to be mesenteric in origin (see below): in the writers' view this merely adds confusion to an already complex terminology. Since this is their normal fate it seems preferable to term these the ventral and dorsal *pars diaphragmatica* of the oesophageal mesenchyme from the outset.



The remaining subdiaphragmatic part of the septum 'transversum', as indicated, is designated the *pars mesenterica* and initially forms a sagittally placed ventral 'mesentery' for the foregut. Although a continuous sheet, thick subregions are recognized: a brief *ventral meso-oesophagus*, continuing into a more extensive *ventral mesogastrium*, the latter merging into a *ventral mesoduodenum* that has a caudal free border. Many (the majority of) accounts ignore these subregions and simply equate all derivatives of the ventral *pars mesenterica* as those of **the ventral mesogastrium**. Some confusion may occur, however, particularly with respect to the development and definitive courses of the main biliary ducts, hepatic artery, portal vein and attendant lymphatics and nerves, and to the hepatic and gastric serosae and mesenteries. As indicated, the mesenchymal modifications are correlated with the asymmetric growth patterns of the stomach, duodenum and liver (see pp. 183📖, 186📖 and below).

Abdominal Foregut Dorsal Splanchnopleuric Mesenchyme

Abdominal foregut dorsal splanchnopleuric mesenchyme commences rostrally as an oesophageal *dorsal pars diaphragmatica* which, as indicated, contributes a narrow midline strip extending between the oesophageal hiatus and the aortic orifice. The remainder, the *dorsal pars*


mesenterica, again initially forms a midline sagittal dorsal septum of thick mesenchyme; its subregions (corresponding to the ventral ones) and parts of one continuous mesenchymal block are: a brief *dorsal meso-oesophagus*, a more extensive *dorsal mesogastrium* and a *dorsal mesoduodenum*. Their modifications are even more extensive than those of the ventral mesenteries and also have the additional complications of the developing pancreas and spleen. Further, many accounts ignore the subregions and collectively dub them **the dorsal mesogastrium**. This may lead to (perhaps minor) misplacement of the anlage of certain organs; however, the siting of the initiation of the lesser sac of peritoneum in the dorsal mesogastrium is both incorrect and confusing.

Caval Fold




The caval fold is a linear eminence with divergent rostral and caudal ends that passes from the upper abdominal to the lower thoracic region and protrudes from the dorsal wall of the pleuroperitoneal canal. Cranially it becomes continuous, lateromedially, with the root of the pulmonary anlage and pleural coelom, the pars pericardialis of the septum transversum and the retrocardiac mediastinal mesenchyme. Caudally it forms an arch with *dorsal* and *ventral* horns; the dorsal merges with the primitive dorsal mesentery and the mesonephric ridge (and associated gonad and suprarenal); the ventral horn is confluent with the dorsal surface of the septal mesenchyme. Thus the fold is a zone where intestinal, mesenteric, intermediate, hepatic, pericardial, pulmonary and mediastinal mesenchymes meet and blend, and may justifiably be compared with the junctional zones of mesenchyme described elsewhere (p. 151 ). Contributions from septal mesenchyme are considered of particular importance by some authorities (Kanagasuntheram 1957 ). It provides a mesenchymal route for the upper abdominal, transdiaphragmatic and transpericardial parts of the inferior vena cava; it is also prominent in the development of parts of the liver, lesser sac of peritoneum and certain mesenteries. The left fold regresses whereas the right fold enlarges rapidly.

Lesser Sac

The lesser sac (*definitive bursa omentalis*) has already been mentioned in terms of its general disposition and communication with the greater sac. Unfortunately considerable variation exists between the terminologies adopted by different authors, both during ontogeny and in adult topography. Development of the lesser sac is so intimately interlocked with additional ontogenetic features, particularly of the liver and stomach, their mesenteries, and secondary extensions of the greater sac, that these must receive brief mention.

Invasion of the ventral pars mesenterica by the hepatic and (transiently) the ventral pancreatic rudiment, followed by trifurcation of the primary hepatic anlage into right and left submasses and a caudal pars cystica, has been described (p. 187 ). Hepatic growth on the two sides is approximately equal in early fetal months, and the liver mass almost fills the ventral mesenchyme; but thick, short blocks of mesenchyme intervene between the liver and the foregut, diaphragm and supra-umbilical ventral body wall. Removal of the viscera at these symmetric stages reveals the cut edges of the mesothelium bordering the coelom and enclosing the wide areas of mesenchymal continuity. The latter has a large roughly circular central area with

numerous tapering projections towards the abdomen: the obliquely cut mesenteries, caval folds, mesonephric and attendant ridges and the lateral rim of the pleuroperitoneal canals. The whole bears some resemblance to an inverted coronet, hence the name coronary ligament of the liver. (With growth and maturity the restricted use of the name is much less apposite.) Much of the mesenchymal mass surrounding the liver develops cavities that coalesce and open into the general coelomic cavity as extensions of the greater (and lesser) sacs of peritoneum. Thus almost all the ventrosuperior, visceral and some of the posterior aspects of the liver become peritonealized. The process involving the greater sac continues over the right lobe and ceases when the future superior and inferior layers of the coronary ligament and the right triangular ligament are defined. Those, plus a medial boundary provided by an extension of the lesser sac, enclose the 'bare area' of the liver where loose areolar tissue of septal origin persists. Due to asymmetric liver growth the same processes affecting the left lobe result in the smaller left triangular ligament. Where the superior layers of the coronary and left triangular ligaments meet they continue as a (bilaminar) ventral mesentery attached to the ventrosuperior aspects of the liver; its parietal attachment has a slight inclination to the right as it crosses the ventral diaphragm and then descends to the umbilicus. Its umbilicohepatic free caudal border, somewhat arched, carries the left umbilical vein (or, postnatally, the fibrous obliterated vein or *ligamentum teres*, with fine calibre para-umbilical veins); the whole structure constitutes the *falciform ligament*. Most of the latter may be considered the final ventral part of the ventral mesogastrium; its free border, however, has a ventral mesoduodenal origin.

As stated, the endodermal subdiaphragmatic foregut of the early embryo is a tube of almost uniform calibre, but with a slight fusiform gastric dilatation with a minimal degree of side-to-side flattening, encased in thick splanchnopleuric mesenchyme and splanchnopleuric coelomic epithelia (mesothelium). The mesenchyme is continuous with the primitive ventral and dorsal mesogastria and these indicate the sagittal positions of the *primitive* ventral and dorsal borders of the stomach. Many accounts state that the development of a ventrally directed concave *lesser curvature* and a more rapidly growing dorsocranially directed convex *greater curvature* and incipient *fundus* occur while the anlage is still sagittal. Thereafter, the whole miniature organ and attached mesogastria are supposed to physically rotate through almost 90° (see p. 190 ) . On this view the original left surface becomes ventrosuperior and the primitive dorsal border is directed to the left as the greater curvature. The interpretation of various mesenteric and neurovascular sequelae were based on these assumptions. Inspection of closely-spaced series of embryos (Kanagasuntheram 1957 , 1960 ) led to the suggestion that the morphogenetic changes in both stomach and duodenum and their attendant mesenteries were mainly, if not wholly, due to patterned differential growth rates and mesenchymal cleft formation and cleavage. Thus the stomach, initially a tube with slight lateral flattening, developed a linear zone of high proliferation along the length of the central part of its (original) left lateral wall. At first it became triangular in transverse section, with rounded angles and apex pointing to the left; further growth resulted in an elliptical profile. The ends of the sectional ellipse corresponded to the curvatures: the end directed to the right, and a little ventrally, to the lesser curvature and justifiably regarded as a modified primitive ventral border; the left end to the greater curvature, an entirely *new formation*. For a while the two primitive mesogastria are **both** attached near the emerging lesser curvature (the sites of the primitive dorsal and ventral borders). The profound changes involved in reaching the definitive state only supervene with the development of the

lesser sac.



The *lesser sac* is the first indicated by the appearance of multiple clefts in the para-oesophageal mesenchyme on both left and right aspects of the oesophagus. Although they may become confluent, the left clefts are transitory and soon atrophy. The right clefts merge to form the *right pneumato-enteric recess* that extends from the oesophageal end of the lesser curvature as far as the caudal aspect of the right lung bud. At its gastric end it communicates with the general peritoneal cavity and lies **ventrolateral** to the gut; more rostrally it lies directly **lateral** to the oesophagus. It is **not**, as commonly stated, a simple progressive excavation of the right side of the dorsal mesogastrium. The right pneumato-enteric recess undergoes further extension, subdivision and modification mentioned below. From its caudal end a second process of cleft and cavity formation occurs producing the *hepato-enteric recess* that thins and expands the splanchnopleure between the liver and the stomach and proximal duodenum, and also reaches the diaphragm. The resulting, structurally bilaminar, mesenteric sheet is the *lesser omentum* and is derived from the small meso-oesophagus, the major part from the ventral mesogastrium, and the reduplicated strip including the free border from the ventral mesoduodenum. As differential growth of the duodenum occurs, the biliary duct is repositioned and most of the duodenum becomes sessile; the duodenal attachment of the free border and a continuous neighbouring strip of the lesser omentum becomes confined to the upper border of a short segment of its superior part. The free border, with contrasting growth and positioning of its attached viscera, gradually changes from the horizontal to the vertical. It carries the bile duct, portal vein and hepatic artery, and its hepatic end is reflected around the porta hepatis; an alternative name for this part of the lesser omentum is the *hepatoduodenal ligament*, and it forms the anterior wall of the epiploic foramen. The floor of the foramen is the initial segment of the superior part of the duodenum mentioned above, its posterior wall is the peritoneum covering the immediately subhepatic part of the inferior vena cava, and its roof the peritonealized caudate process of the liver. That major part of the lesser omentum from the lesser gastric curvature passes in an approximately coronal plane to reach the floor of the increasingly deep groove for the ductus venosus (postnatally the *ligamentum venosum*) on the hepatic dorsum. This part is sometimes called the *hepatogastric ligament*.

The pneumato-enteric recess continues to expand to the right into the substance of the caval fold, ceasing near the left margin of the hepatic part of the inferior vena cava; the latter remains extraperitoneal, crossing the base of the now roughly triangular bare area of the liver (encompassed by coronary and right triangular ligaments) and this new expanded line of reflexion. With closure of the pleuroperitoneal canals the rostral part of the right pneumato-enteric recess is sequestered by the diaphragm but often persists as a small serous sac in the right pulmonary ligament. The remaining caval fold mesenchyme to the left of the inferior vena cava, and forming the right wall of the upper part of the lesser sac, becomes completely invaded by embryonic hepatic tissue and is transformed into the caudate lobe of the liver. This smooth, vertically elongate mass projects into the cavity of the lesser sac, and both its posterior and much of its anterior surfaces are peritonealized because of the increasing depth of the groove for the ductus venosus and the attachment of the lesser omentum to its *floor*. The narrow isthmus of the *caudate process* connects its right inferior angle to the rest of the right lobe (and is the roof of the epiploic foramen). The parts of the lesser sac thus far described above and to the right

of the lesser curvature of the stomach have received a plethora of names by different investigators; sometimes these stem from changing or contrasting views of ontogeny. A few prominent ones will be mentioned. Some regard the right rim only of the structures already listed as constituting the boundaries of the epiploic foramen. The narrow channel, a few centimetres long, passing to the left, and coextensive with the caudate process and peritonealized part of the initial duodenal segment, is called the *vestibule* of the lesser sac (but see below). The remaining, much more extensive part above the lesser curvature is termed, variously, the *upper (superior) part* (recess) of the lesser sac, the *hepatic part* of the lesser sac or finally the *hepato-enteric part of the pneumato-enteric recess*. Some authorities group all the foregoing under the general term *vestibule*; they are intimately related to the diaphragm and caudate lobe of the liver and coextensive with the lesser omentum. Varying use of the name vestibule partly reflects earlier, mistaken views of the ontogeny of the upper and lower parts of the lesser sac (about to be described). Much of the upper part was considered to be merely a part of the general peritoneal coelom 'captured' by differential growth and omental changes of plane, whereas fundamentally different mechanisms of excavation were considered to apply to the lower part, the '*true*' *omental bursa* of some authors. Current evidences suggest that the **same** array of developmental mechanisms, with only quantitative regional differences, apply to **all** coelomic regions. The general name omental bursa should be used with caution; both upper and lower parts have walls that are partly omental (lesser and greater omenta respectively); also neither part is functionally more nor less bursal than other coelomic regions—peritoneal, pleural or pericardial. Confusion stemming from the term 'recess' firmly established in embryology but, with different connotations, used sporadically in adult topography has been mentioned (p. 1744📖). Thus, the upper (superior) and lower (inferior) parts of the lesser sac seem preferable terms. Ultimately the junction between upper and lower parts is oblique, curving upwards as it passes from right to left; ventrally lies the gastric lesser curvature, dorsally the body of the pancreas. The left limit is a curved ridge of mesenchyme (future left *gastropancreatic fold*) carrying the left gastric artery and the right a curved ridge (future right *gastropancreatic fold*) carrying the common hepatic artery.

The *lower (inferior) part of the lesser sac* commences development at about 8–9 mm CR length—at this stage the early pneumato-enteric and hepato-enteric recesses are well established. Differential gastric growth is progressing (p. 183📖) giving an elliptical transverse sectional profile, with a right-sided lesser curvature, corresponding to the original ventral border of the gastric tube, to which the lesser omental gastric part of the ventral mesogastrium remains attached. As indicated, the greater curvature is a new formation, rapidly expanding, with its convex profile projecting mainly to the left, but also rostrally and caudally. It was emphasized that the original dorsal border of the gastric tube now traverses the dorsal aspect of the expanding rudiment, curving along a line near the *lesser* curvature; here, transiently, the *primitive* dorsal mesogastrium is attached. The latter blends with the thick layer of compound gastric mesenchyme clothing the posterior aspect and greater curvature of the miniature stomach; because of its thickness, the mesenchyme projects rostrally, caudally, and particularly to the left, beyond the 'new' greater curvature of the endodermal lining of the stomach. (It may be noted that during these stages the geometries, and hence sectional profiles, of tissue strata and cavities are complex and change rapidly with varying levels of histological section and with time. Thus only a brief summary of some salient points can be attempted here. For details consult Kanagasuntheram 1957📖.) The processes already described in relation to the ventral mesenteries


now supervene. Multiple clefts appear at various loci in the mesenchyme, with local mesenchyme to epithelial transition; each group of clefts rapidly coalesce to form (transiently) isolated closed spaces. The latter, by a continuation of the mechanism, soon join with each other and with the preformed upper part of the lesser sac, the newly formed epithelia joining the coelomic epithelium. The initial loci involve, firstly, the compound posterior gastric mesenchyme nearer the lesser curvature and along its zone of blending with the primitive dorsal mesogastrium; secondarily, in the dorsal mesoduodenum; thirdly, independently in the caudal rim where greater curvature mesenchyme and dorsal mesogastrium blend. As these cavities become confluent and their 'reniform' expansion follows, matches and then exceeds that of the gastric greater curvature, there are some major sequelae. The primitive dorsal mesogastrium increases in area not only by intrinsic growth, but as cavitation proceeds, by substantial additions from the dorsal lamella separated by cleavage of the posterior gastric mesenchyme: together these may conveniently be called the *secondary dorsal mesogastrium*. The *gastric attachment* of the latter changes as a set of somewhat spiral lines, longitudinally disposed, that move, with time, from near the lesser curvature towards, and finally reaching, the definitive greater curvature. The *parietal* mesogastral and (cleaving) mesoduodenal *attachment* remains, for a time, in the dorsal midline, but subsequently undergoes profound changes. With the confluence of the cavities that collectively form the lower part of the lesser sac, its communication with the upper part, corresponding to the lesser gastric curvature and right and left gastropancreatic folds, becomes better defined. **Ventral** to the lower part of the cavity lies the postcleavage splanchnopleure covering the postero-inferior surface of the stomach and a short proximal segment of the duodenum. This ventral wall is continued beyond the greater curvature and duodenum as the splanchnopleuric strip of visceral attachment of the secondary dorsal mesogastrium and mesoduodenum. The radial width of the strip is relatively short rostrally (gastric fundus) and gradually increases along the descending left part of the greater curvature; it is longest throughout the remaining perimeter of the greater curvature as far as the duodenum, and this prominent part shows continued marginal (caudoventral and lateral) growth with extended internal cavitation (its walls constituting the expanding *greater omentum*). The **margins** of the cavity of the inferior part of the lesser sac are limited by the reflexed edges of the ventrally placed strata derived from the secondary dorsal mesogastrium just described. These converge, forming the splanchnopleuric dorsal wall, which is initially 'free' throughout except at its midline dorsal root. At roughly midgastric levels, encased in this dorsal wall, the pancreatic rudiment grows obliquely, its tail ultimately reaching the left limit of the lesser sac at the level of the junction between gastric fundus and body. (It may be mentioned that many earlier accounts held that the lower part of the lesser sac stemmed from a simple excavation of the dorsal mesogastrium—the pancreatico-enteric recess—with subsequent further extensions. This view has been discarded here.)

Centred on the dorsal mesogastral region towards which growth of the pancreatic tail is directed, the anlage of the spleen appears in the coelomic epithelium and subjacent mesenchyme. As it expands into the upper left hypochondriac part of the *greater* sac, it retains its neurovascular pedicle and serous tunic, both of dorsal mesogastral origin (pp. 1438 , 1739 ).

The greater omentum and its contained extension of the lesser sac continue to grow both

laterally, and particularly caudoventrally, covering and closely applied to the transverse mesocolon, transverse colon and inframesocolic and infracolic coils of small intestine. At this stage the quadrilaminar nature of the dependent part of the greater omentum is most easily appreciated. It will be recalled that 'simple' mesenteries, for example the mesentery of the jejuno-ileum, are bilaminar in that they possess two mesothelial surfaces (splanchnopleuric coelomic epithelium) enclosing a connective tissue core (from splanchnopleuric mesenchyme) which bears blood and lymphatic vessels, lymph nodes, nerves, adipose tissue and other cell varieties. In the greater omentum, the gastric serosa covering its postero-inferior surface (single mesothelium) and the anterosuperior serosa (single mesothelium) converge, meeting at the greater curvature (and initial segment of the duodenum). The resulting bilaminar mesentery continues as the anterosuperior (or 'descending') stratum of the omentum which, on reaching the omental margins, is reflexed and 'returns' (or 'ascends') as its posterior bilaminar stratum to its parietal root. The two bilaminar strata are initially in fairly close contact caudally, but separated by a fine, fluid-containing, cleft-like extension of the lower part of the lesser sac. The posterior mesothelium of the posterior stratum makes equally close contact with the anterosuperior surface of the transverse colon (starting at its *taenia omentalis*) and with its transverse mesocolon.

At this stage, and subsequently, it is convenient to designate the lower part of the lesser sac as consisting of three *subregions*: *retrogastric*, *perigastric* and *greater omental*. The names are self-explanatory but their confines are all modified by various factors. Two phenomena are particularly prominent: gastric 'descent' relative to the liver, and fusion of peritoneal layers with altered lines of reflexion, adhesion of surfaces and loss of parts of cavities.

After the third month the hepatic growth diminishes, particularly the left lobe, and the whole organ recedes into the upper abdomen; meanwhile the stomach elongates and some descent occurs, despite its relatively fixed cranial and caudal ends. This causes the angular flexure of the stomach that persists postnatally: the concavity of the lesser curvature is now directed more precisely to the right, the lesser omentum is more exactly coronal and its free border vertical, ventral to the liver; the free border of the falciform ligament passes steeply rostr dorsally from umbilicus to liver. The mesenchymal dorsal wall of the lower part of the lesser sac, crossed obliquely by the growing pancreas, has hitherto remained free, with its original dorsal midline root. Substantial areas now fuse with adjacent peritonealized surfaces of retroperitoneal viscera, the parietes, or another mesenteric sheet or fold. In the latter case there occurs a variable loss of their apposed mesothelia and some continuity of their mesenchymal cores, but they remain surgically separable and no vascular anastomosis develops across the interzone. Above the pancreas the posterior secondary dorsomesogastric wall of the sac becomes closely applied to the peritoneum covering the posterior abdominal wall and its sessile organs—the diaphragm, much of the left suprarenal gland, the ventromedial part of the upper pole of the left kidney, the initial part of the abdominal aorta, the coeliac trunk and its branches, and other vessels, nerves, and lymphatics. Their peritoneal surfaces fuse and, with some tissue loss, a **single** mesothelium covering these structures remains, intercalated as a new secondary dorsal wall for this part of the lesser sac. The pancreas, as indicated (p. 186 ) , grows from the duodenal loop, penetrating the substance of the dorsal mesoduodenum and secondary dorsal mesogastrium, their mesenchymes and mesothelia initially clothing its whole surface, except where there exist peritoneal lines of reflexion. Its posterior peritoneum becomes closely applied to that covering all the posterior


abdominal wall structures it crosses (prominently: the inferior vena cava, abdominal aorta, splenic vein, superior mesenteric vessels, inferior mesenteric vein, portal vein, left renal vessels, the caudal pole of the left suprarenal, a broad ventral band on the left kidney and various muscles, etc; see p. 1790^[1]). The intervening peritoneal mesothelia fuse and atrophy, the mesenchymal cores forming fascial sheaths and septa. The pancreas is now sessile and the peritoneum covering the upper left part of its head, neck and anterosuperior part of its body forms the central part of the dorsal wall of the lesser sac. The pancreatic tail remains peritonealized by a persisting part of the secondary dorsal mesogastrium as it curves from the ventral aspect of the left kidney towards the hilum of the spleen. (The infracolic parts of the pancreas are covered with greater sac peritoneum.) In the greater omental subregion of the lower part of the lesser sac two contrasting forms of mesenteric adhesion occur. The posterior 'returning' bilaminar stratum of the omentum undergoes partial fusion with the peritoneum of the transverse colon (at the taenia omentalis) and with its mesocolon. (They provide a great increase in the functional area of the stomach 'bed', p. 1755^[2]; also they remain surgically separable and no anastomosis occurs between omental and colic vessels.) In fetal life the greater omental cavity extends to the internal aspect of the lateral and caudal edges of the omentum. However, postnatally a slow but progressive fusion of the internal surfaces occurs with obliteration of the most dependent part of the cavity; this proceeds rostrally and, when mature, the cavity does not usually extend appreciably beyond the transverse colon. (The other fusions described above follow different patterns.) Transverse mesocolon–greater omentum fusion commences early while the umbilical hernia of the midgut has not returned; it is initiated between the right margin of the early greater omentum and near the root of the *presumptive* mesocolon, later spreading to the left. The pancreas becomes sessile mediolaterally (i.e. head, followed by neck and body from right to left). Paradoxically, in the suprapancreatic region, the direction of the process of fusion is reversed, progressing lateromedially from left to right. These events are wholly in accord with the numerous variations and anomalous visceral positioning and mesenteric arrangements that have been observed and recorded.

Correlated with the many facets of development of the lesser sac and its associated viscera, the original dorsal midline attachment to the parietes of the foregut dorsal mesentery is profoundly altered. However, despite the extensive areas of fusion, virtually the whole of the gastric greater curvature (other than a small suboesophageal area) and its topographical continuation, the inferior border of the first 2–3 cm of the duodenum, retain true mesenteric derivatives of the secondary dorsal mesogastrium and its continuation, the dorsal mesoduodenum. Thus, although regional names are given to assist identification and description, it must be emphasized that they are merely subregions of one continuous sheet; a short description of their 'root' or parietal line of reflexion is also given.


The upper (oesophagophrenic) part of the *lesser* omentum arches across the diaphragm and as this bilaminar mesentery approaches the oesophageal hiatus its laminae diverge, skirting the margins of the hiatus. They then descend for a limited distance and variable inclination, to enclose reciprocally-shaped areas on the dorsum of the gastric fundus and diaphragm. The area may be roughly triangular to quadrangular; it contains areolar tissue and constitutes the *bare area of the stomach* or, when large, the *left extraperitoneal space*. Its right lower angle is the base of the left gastropancreatic fold; its left lower angle reconstitutes the bilaminar mesentery.


The root of the latter arches downwards and to the left across the diaphragm and suprarenal and gives the *gastrophrenic ligament* to the gastric fundus. Continuing to arch across the ventral surface of the upper part of the left kidney, its layers part to receive the pancreatic tail and initially extend to the hilum of the spleen as the *splenorenal (lienorenal) ligament*. The left half of this bilaminar 'ligament' provides an almost complete peritoneal tunic for the spleen (as indicated, projecting into the greater sac) then, reuniting with its fellow at the opposite rim of the splenic hilum, continues to the next part of the gastric greater curvature as the *gastrosplenic ligament*. The remaining major (perhaps two-thirds) of the gastric greater curvature and its short duodenal extension are the visceral attachment of the anterior, 'descending', bilaminar stratum of the greater omentum. Its returning, posterior, bilaminar stratum continues to its parietal root; the latter extends from the inferior limit assigned to the splenorenal ligament, continuing to curve caudally and to the right along the anterior border of the body of the pancreas, immediately cranial to the line of attachment of the transverse mesocolon. Crossing the neck of the pancreas, the same curve is followed for a few centimetres on to the gland's head; the omental root is then sharply recurved cranially and to the left, soon to reach the inferior border of the duodenum. Thus it reaches that part of the lesser sac provided by cleavage of the dorsal mesoduodenum from the greater sac, entering the epiploic foramen and traversing the epiploic canal between the caudate hepatic process and proximal duodenum, the lower right angle of the hepato-enteric recess, and crossing the right gastro-pancreatic fold, then descending behind the proximal duodenum to enter the right marginal strip enclosed by the greater omentum.



Urinary and Reproductive Systems

The urinary and reproductive organs are developed from *intermediate mesenchyme* (p. 155 ) and are intimately associated with one another especially in the earlier stages of their development. The urinary system develops ahead of the reproductive or genital system.

Urinary System

The intermediate mesenchyme is found longitudinally placed in the trunk, subjacent to the somites (in the folded embryo), at the junction between the splanchnopleuric mesenchyme adjacent to the gut medially and the somatopleuric mesenchyme subjacent to the ectoderm laterally (3.80 )). Development of the intermediate mesenchyme progresses craniocaudally. In lower vertebrates the intermediate mesenchyme typically develops serial, segmental epithelial diverticuli termed *nephrotomes* each enclosing a cavity, the *nephrocoele*, and communicating with the coelom through a *peritoneal funnel*. The dorsal wall of each nephrotome evaginates as a *nephric tubule* communicating with the nephrocoele via a *nephrostome*. The dorsal tips of the earlier (cranial) developed nephric tubules bend caudally and fuse forming a longitudinal *primary excretory duct*, which grows caudally and curves ventrally to open into the cloaca. The more caudally placed and successively later developed tubules open secondarily into this duct or into tubular outgrowths from it. *Glomeruli*, specific arrangements of capillaries and overlying coelomic epithelium, arise from the ventral wall of the nephrocoele (*internal glomeruli*) or the roof of the coelom adjacent to the peritoneal funnels (*coelomic or external glomeruli*), or in both


situations (3.81 ). An extensive area vascularized as a *glomus* rather than individual glomeruli can be seen in developing reptiles during organogenesis. In avian species external (pronephric, see below) glomeruli can be identified. Clusters of cells appropriately placed in mammalian embryos fail to differentiate into true glomeruli.

It has been customary to regard the renal excretory system as three organs, the *pronephros*, *mesonephros* and *metanephros*, succeeding each other in time and space. The last to develop is retained as the permanent kidney. It is, however, difficult to provide reliable criteria that distinguish them as individual organs or to define their precise limits in the embryos of all animals. A functional pronephros with well-developed podocytes has been identified in chelonian embryos (Collins 1990 ) and in avian embryos (Jacob et al 1977 ); however, a pronephros cannot be distinguished as a separate organ in man. The earliest and most cranially situated nephric tubules are rudimentary, transient and regarded as marking the pronephric region, and the latter merges caudally without clear demarcation into the mesonephros. Human nephrotomes, with cavitation to form nephrocoeles which communicate with the coelom, are restricted to a few segments bordering the rostral limit of the intraembryonic coelom. Cranial to this (pronephric region) the intermediate mesenchyme develops irregular, transient, solid or vesicular balls of cells. Caudal to the level of the eighth to tenth somites the intermediate mesenchyme is termed the *nephrogenic cord*. This is connected at irregular intervals with the coelomic epithelium.

Primary Excretory Duct

This begins in stage 11 embryos of about 14 somites as a solid rod of cells in the dorsal part of the nephrogenic cord. Its cranial end is about the level of the ninth somite and its caudal tip merges with the undifferentiated mesenchyme of the cord. It differentiates before any nephric tubules; when the latter appear it is at first unconnected with them. In older embryos the duct has lengthened and its caudal end becomes detached from the nephrogenic cord to lie immediately beneath the ectoderm. From this level it grows caudally, independent of the nephrogenic mesenchyme, and then curves ventrally to reach the wall of the cloaca. It becomes canalized progressively from its caudal end to form a true duct which opens into the cloaca in embryos at stage 12. (Clearly, up to this stage, the name 'duct' is scarcely appropriate.)

Pronephros

The intermediate mesenchyme becomes visible in stage 10 embryos and the nephrogenic cord is distinguishable when 10 somites are present. The pronephros then is indicated as clusters of cells (rudimentary nephric tubules) in the nephrogenic mesenchyme (cord) (3.81 ). In regions cranial to the primary excretory duct these clusters develop no further. More caudally similar groups of cells appear and these become vesicular. The dorsal ends of the most caudal of these vesicles join the primary excretory duct, their central ends being connected with the coelomic epithelium by cellular strands probably representing rudimentary peritoneal funnels. Glomeruli are not developed in association with these cranially situated nephric tubules, which ultimately disappear. It is doubtful whether external glomeruli develop in human embryos. (For an overview and bibliographies concerning the early development of the human nephros consult

Torrey 1954^[1]; O'Rahilly & Muecke 1972^[2]; O'Rahilly & Muller 1987^[3].)

Mesonephros







From about stage 12 the primary excretory duct is termed the *mesonephric duct* (*Wolffian duct*); it lengthens and soon becomes connected to the cloaca. At this stage mesonephric tubules, developing from the intermediate mesenchyme between somite levels 8–20, begin to connect to the mesonephric duct; caudal to this there is a continuous ridge of nephrogenic mesenchyme to the level of somite 24. The mesonephric tubules are not metameric: there may be two or more mesonephric tubules opposite each somite.

Within the mesonephros, each tubule first appears as a condensation of mesenchyme cells which epithelialize and form a vesicle. One end of the vesicle grows towards and opens into the mesonephric duct, whilst the other dilates and invaginates; the outer stratum forms the *glomerular capsule*, while the inner cells differentiate into *mesonephric podocytes* which clothe the invaginating capillaries to form a glomerulus. The latter are supplied with blood through lateral branches of the aorta (Streeter 1945^[4]). It is estimated that about 70–80 mesonephric tubules and a corresponding number of glomeruli develop. These tubules, however, are not present at the same time and it is rare to find more than 30–40 in an individual embryo, for the cranial tubules and glomeruli develop and atrophy before the development of those situated more caudally.

By the end of the sixth week each mesonephros is an elongated spindle-shaped organ which projects into the coelomic cavity, one on each side of the dorsal mesentery, from the level of the septum transversum to the third lumbar segment. This whole projection is the *mesonephric ridge* (Wolffian body); it develops subregions (see below), and the gonad is developed on its medial surface. There are striking similarities in structure between the mesonephros and the permanent kidney or metanephros, but the former's nephrons lack a segment corresponding to the descending limb of the loop of Henle (Leeson 1957^[5]; Davies & Routh 1957^[6]). The mesonephros is believed to produce urine by stage 17 (O'Rahilly & Muller 1987^[3]). A detailed comparison of the development and function of the mesonephros and metanephros in staged human embryos is lacking. In the chick no significant difference in the renal clearance of plasma sodium, potassium and magnesium ions were noted in 9-day embryos with functional mesonephric kidneys, and 15-day embryos with predominant metanephric kidneys; calcium clearance decreased over this time (Clarke et al 1993^[7]).




In stage 18 embryos (13–17 mm) the mesonephric ridge extends cranially to about the level of rib 9. In both sexes the cranial end of the mesonephros atrophies; in embryos of 20-mm length (stage 19) the organ is found only in the first three lumbar segments, although it may still possess as many as 26 tubules. The most cranial one or two tubules persist as the *rostral aberrant ductules*; the succeeding five or six tubules develop into the *efferent ductules of the testis* and the *lobules of the head of the epididymis* in the male, and the tubules of the *epoöphoron* in the female; the caudal tubules form the *caudal aberrant ductules* and the *paradidymis* in the male, and the *paroöphoron* in the female (see below).

Mesonephric Duct

This runs caudally in the lateral part of the nephric ridge, at the caudal end of which it projects into the cavity of the coelom in the substance of a *mesonephric fold* (3.82 , 83 , 84 , 85 , 86 ). (Subsequently, with the neighbouring formation of the paramesonephric duct, they form the *tubal fold*—see below.) As the mesonephric ducts from each side approach the urogenital sinus the two folds fuse with each other, between the bladder ventrally and the rectum dorsally, forming across the cavity of the pelvis a transverse partition which is somewhat inaptly termed the *genital cord* (3.84 ). In the male the peritoneal fossa between the bladder and the genital cord becomes obliterated, but it persists in the female as the uterovesical pouch. The mesonephric duct itself becomes the canal of the *epididymis*, *ductus deferens* and *ejaculatory duct*. The *seminal vesicle* and the ampulla of the ductus deferens appear as a common swelling at the termination of the mesonephric duct during the end of the third and into the fourth month. This coincides with degeneration of the paramesonephric ducts, though no causal relation between the two has been established. Separation into two rudiments occurs at about 125-mm crown–heel length. The seminal vesicle elongates, its duct is delineated and hollow diverticula bud from its wall. About the sixth month (300-mm crown–heel length) the growth rate of both vesicle and ampulla is greatly increased; the cause is uncertain but may result from increased secretion of prolactin by the fetal or maternal hypophysis, or the effects of placental hormones. The tubules of the prostate show a similar increase of growth rate at the same time. In the female the mesonephric duct is vestigial, becoming the longitudinal duct of the epoöphoron.

Metanephros

Both the pronephros and the mesonephros are linear structures with stacks of tubules arranged along the craniocaudal axis of the embryo. This arrangement results in the production of hypotonic urine. The tubular arrangement in the metanephric kidney is fundamentally different: the tubules are arranged *concentrically* with the loops of Henle directed towards the renal pelvis. This arrangement allows different concentration gradients to develop within the kidney and results in the production of *hypertonic* urine. Thus metanephric nephrons do *not* join with the existing mesonephric duct but with an evagination of that duct which branches *dichotomously* resulting in the particular arrangement of collecting ducts of the metanephric kidney.

The metanephric kidney develops from three sources: an evagination of the mesonephric duct, the *ureteric bud*, and a local condensation of mesenchyme termed the *metanephric blastema* form the nephric structure, while *angiogenic mesenchyme* migrates into the metanephric blastema slightly later to produce the glomeruli and vasa recta (Grobstein 1955 ; Saxén et al 1968 ; Saxén 1970 ). It may also be the case that innervation is necessary for metanephric kidney induction (see below).

There is an epithelial/mesenchymal interaction between the duct system and the surrounding mesenchyme in both mesonephric and metanephric systems. However, whereas in the mesonephric kidney development proceeds in a *craniocaudal progression*, with cranial nephrons degenerating before caudal ones are produced, in the metanephric kidney a proportion of the

mesenchyme remains as stem cells which continue to divide and enter the nephrogenic pathway later when the individual collecting ducts lengthen. Thus the temporal development of the metanephric kidney is patterned *radially*, with the outer cortex being the last part to be formed. Generally the interactions in metanephric kidney development are as follows: the ureteric bud undergoes a series of *bifurcations* within the surrounding metanephric mesenchyme, forming smaller *ureteric ducts*. At the same time the metanephric mesenchyme condenses around the dividing ducts into smaller condensations. These form *S-shaped clusters* which transform into epithelia and fuse with the ureteric ducts at their distal ends. Blood vessels invade the proximal ends of the S-shaped clusters to form the *vascularized glomeruli*.

The ureteric bud bifurcates when it comes into contact with the metanephric blastema as a result of local extracellular matrix molecule synthesis by the mesenchyme. In metanephric culture, incubation of fetal kidneys in β -D-xyloside, an inhibitor of chondroitin sulphate synthesis, dramatically inhibits ureteric bud branching. Both chondroitin sulphate proteoglycan synthesis and chondroitin sulphate glycosaminoglycan processing are necessary for the dichotomous branching of the ureteric bud (Fouser & Avner 1993). Subsequent divisions of the ureteric bud and the mesenchyme form the gross structure of the kidney with *major* and *minor calyces*, the distal branches of the ureteric ducts will form the *collecting ducts* of the kidney. As the collecting ducts elongate the metanephric mesenchyme condenses around them. An adhesion molecule, syndecan, can be detected between the mesenchymal cells in the condensate. The cells cease expression of N-CAM, fibronectin and collagen I and commence production of L-CAM (an adhesion molecule also called E cadherin) and the basal lamina constituents laminin and collagen IV. The mesenchymal clusters thus convert to small groups of epithelial cells which undergo complex morphogenetic changes. Each epithelial group elongates, forms a comma-shaped, then an S-shaped body which elongates further. It then fuses to a branch of the ureteric duct at its distal end while expanding as a dilated sac at the proximal end. The sac involutes with local cellular differentiation such that the outer cells become the *parietal glomerular cells*, while the inner ones become *visceral epithelial podocytes*. The podocytes develop in close proximity to invading capillaries which derive from *angiogenic mesenchyme* outside the nephrogenic mesenchyme (Eklom et al 1982); this third source of mesenchyme produces the *endothelial* and *mesangial cells* within the glomerulus. Both the metanephric derived podocytes and the angiogenic mesenchyme produce fibronectin and other components of the glomerular basal lamina. The isoforms of type IV collagen within this layer follow a specific programme of maturation which occurs as the filtration of macromolecules from the plasma becomes restricted (Bard & Woolf 1992).

Platelet derived growth factor (PDGF) β -chain and the PDGF receptor β -subunit (PDGFR β) have been detected in developing human glomeruli between 54 and 105 days gestation. PDGF β -chain is localized in the differentiating epithelium of the glomerular vesicle during its comma and S-shaped stages, while PDGFR β is expressed in the undifferentiated metanephric blastema, vascular structures and interstitial cells. Both PDGF β -chain and PDGFR β are expressed by mesangial cells; this may promote further mesangial cell proliferation.

Metanephric mesenchyme will develop successfully in vitro making experimental perturbation

of kidney development comparatively easy to evaluate. Early experimental studies demonstrated that other mesenchymal populations and also spinal cord were able to induce ureteric bud division and metanephric development. However, it has been demonstrated that nerves enter the developing kidney very early, via the ureter (Sariola et al 1989^[4]). If developing kidney rudiments are incubated with antisense oligonucleotides that neutralize nerve growth factor receptor (NGF-R) mRNA, nephrogenesis is completely blocked, leading to the conclusion that metanephric mesenchyme induction is a response to innervation (Sariola et al 1991^[4]). The powerful inductive effect of the spinal cord on metanephric mesenchyme may be demonstrating this same phenomenon.

All stages of nephron differentiation are present concurrently in the developing metanephric kidney. Antigens to the brush border of the renal tubule appear when the S-shaped body has formed. Tubules displaying this marker appear first in the inner cortical area.

At an early stage the metanephric kidney is **lobulated**, a condition which persists through fetal life but disappears during the first year after birth. Varying degrees of lobulation, however, on occasion, persist throughout life. The growth of left and right kidneys are well matched during development. Fetal kidney volume increases most during the second trimester in both sexes. However, in the third trimester male fetuses show greater values for renal volume than female fetuses (Sampaio 1992^[4]). The reason for this sex difference in renal development remains unknown; however, it has been shown that kidneys from male rats are larger than those from females, attributed to both cellular hyperplasia and hypertrophy (Jean-Faucher et al 1987^[4]), and male fetuses are generally larger than females from 12 weeks postconception (Pedersen 1980^[4]).

Endocrine Development of the Kidney

In addition to its function as an excretory organ, the kidney is an endocrine organ producing hormones acting at sites within and without the kidney. The fetal kidney functions in utero producing *amniotic fluid*; however, *homeostasis* prior to birth is controlled by the *placenta*. Premature babies of less than 36 weeks have immature kidneys with incomplete differentiation of the cortical nephrons compromising their ability to maintain homeostasis. The problems of immaturity are further compounded by the effects of hypoxia and asphyxia which modify renal hormones; also the use of artificial ventilation has effects on renal haemodynamics and the renal hormones.

Hormones produced by the kidney are concerned with renal haemodynamics; they include the renin-angiotensin system, renal prostaglandins, the kallikrein-kinin system, and renal dopamine. *Renin* is found in both the smooth muscle cells of arterioles, interlobular arteries and branches of the renal artery, although it has also been described in the distal convoluted tubule cells; *kallikrein* has been demonstrated in rat fetal kidney (urinary kallikrein is lower in human newborns than in later life); *prostaglandins* have been demonstrated in the renal medulla and in large amounts in the renal tubule; *renal dopamine* has been identified in two sources, from the dopaminergic nerves and predominantly from the enzymatic conversion of levodopa (L-dopa) to dopamine in the early segments of the proximal convoluted tubule. Other hormones have been

identified within the kidney; an antihypertensive lipid is produced in the interstitial cells of the renal medulla, and it also appears that histamine and serotonin can be synthesized by the kidney. Growth factors produced by human embryonic kidney cell include erythropoietin, interleukin β and transforming growth factor-beta. The presence of erythropoietin and interleukin β in human embryonic kidney cells stimulates megakaryocyte maturation (Withy et al 1992^[4]). For a comprehensive account of this aspect of kidney development see Ballie (1992^[4]).

Ascent of the Kidney

When it first appears, the metanephric renal rudiment is sacral but, as the ureteric outgrowth lengthens, it becomes positioned more and more cranially so that when the embryo has a length of some 13 mm its expanded pelvis lies on a level with the second lumbar vertebra. During this period the ascending kidney receives its blood supply *sequentially* from arteries in its immediate neighbourhood, e.g. the middle sacral and common iliac arteries; the definitive *renal artery* is not recognizable until the beginning of the third month. It arises from the most caudal of the three suprarenal arteries, all of which represent persistent mesonephric or lateral splanchnic arteries (p. 318^[4]). Additional renal arteries are by no means uncommon. They may enter at the hilum or at the upper or lower pole of the gland, and they also represent persistent mesonephric arteries.

Anomalies of the Urinary System

Anomalies of the urinary system are relatively common (3% of live births). *Renal agenesis* is the absence of one or both kidneys. In unilateral renal agenesis, the remaining kidney undergoes compensatory hypertrophy producing a nearly normal functional mass of renal tissue. Problems with kidney ascent can result in a *pelvic kidney*, or the kidneys may fuse together at their caudal poles resulting in a *horseshoe kidney* which cannot ascend out of the pelvic cavity because of the proximity to the inferior mesenteric artery which prevents further migration.

Several Hox genes and the Pax 2 gene have been identified in both developing and adult kidney associated with the collecting ducts and ureter. However, this area of research is still in its developmental stage. Genes associated with maldevelopment of the kidney have received further study to date, those associated with Wilm's tumour and renal cystic disease especially (see Bard & Woolf 1992^[4]; Fouser & Avner 1993^[4]). Many varieties of cystic renal disease occur, and a number of different classifications have been proposed. For long it has been held that renal cysts in most, if not all, instances result from vesicular cell clumps retained after failure of fusion between the tips of branches from the ureteric diverticulum on the one hand, and metanephrogenic cap tissue on the other. Such a view is no longer considered tenable. It has been demonstrated, convincingly, that the cyst-like formations are wide dilatations of a part of otherwise continuous nephrons (Moffat 1982^[4]). *Adult polycystic renal disease*, the commonest form, is inherited as an autosomal dominant; in this condition the dilatations may affect any part of the nephron, from Bowman's capsule to collecting tubules. Less common is *infantile cystic renal disease*, inherited as a recessive trait where the proximal and distal tubules are dilated to some degree but the collecting ducts are grossly affected.

With the routine use of ultrasound as an aid to in utero diagnosis of abnormalities, it has been shown that of the prevalence of 1–2 abnormal fetuses per 1000 ultrasound procedures, 20–30% are anomalies of the genitourinary tract. Such abnormalities can be detected as early as 12–15 weeks gestation. However, the decision to be made after such a diagnosis is by no means clear. Urinary obstruction is considered an abnormality yet transient modest obstruction is considered a normal component of the canalization of the urinary tract and has been reported in 10–20% of fetuses in the third trimester. A delay in canalization or in the rupture of the cloacal membrane can produce such a dilatation; similarly, the closure of the urachus at 32 weeks may be associated with high-resistance outflow for the system again resulting in transient obstruction. The degree to which obstruction may cause renal parenchymal damage cannot be assessed in a developing kidney which may have primary nephrogenic dysgenesis (Grupe 1987📖).

The volume of *amniotic fluid* is used as an indicator of renal function. Too little amniotic fluid is termed *oligohydramnios*, too much, *hydramnios*. Although variation in the amount of amniotic fluid may suggest abnormalities of either the gut or kidneys, it is not always possible to correlate even severe oligohydramnios with renal dysfunction. There is an important relationship between the volume of amniotic fluid, lung development and maturity; oligohydramnios has been shown to be associated with pulmonary hypoplasia (see p. 181📖, Lung development).

Ureter

Further development of the ureter has attracted less attention than that of the kidney. The ureteric wall is highly permeable at an early stage (5 mm); its lumen becomes obliterated later (13–22 mm), to be subsequently recanalized. Both processes begin at intermediate levels of the ureter and proceed cranially and caudally. The recanalization is not associated with metanephric function, but possibly with the rapid elongation of the ureter in conformity with embryonic growth. Two fusiform enlargements appear at the lumbar and pelvic levels of the ureter; the lumbar enlargement during the fifth month, the pelvic not until the ninth month (the latter is inconstant). As a result the ureter shows a constriction at its upper end (*pelviureteric region*) and another as it crosses the pelvic brim. A third narrowing is always present at its lower end and is related to the growth of the bladder wall.

At first the caudal connection of the ureter is to the dorsomedial aspect of the mesonephric duct but, owing to differential growth, the connection becomes lateral to the duct. Thereafter the caudal end of the duct becomes incorporated in the developing bladder, and the orifice of the ureter opens separately into the bladder on the lateral side of the opening of the duct. Later the two orifices become separated still further and, although the ureter retains its point of entry into the bladder, the mesonephric duct opens into that part of the urogenital sinus which subsequently becomes the prostatic urethra.

Reproductive System



There are essentially four different cell lineages which contribute to the gonads:

- *proliferating coelomic epithelium* on the medial side of the mesonephroi
- underlying *mesonephric mesenchyme*
- invading *angiogenic mesenchyme* already present in the mesonephroi
- *primordial germ cells*, derived from the epiblast very early in development, which migrate later from their sequestration in the allantoic wall.

The *genital ducts* possess an external serosa derived from coelomic epithelium, a smooth muscle muscularis derived from underlying mesenchyme (similarity of the latter to splanchnopleuric mesenchyme has not yet been investigated), and an internal mucosa from either the *mesonephric duct* or from an invaginated tube of coelomic epithelium which forms the *paramesonephric* or *Mullerian duct*. The layers are invaded by angiogenic mesenchyme and by nerves.

Mesonephric and *paramesonephric (Mullerian)* ducts are produced in all embryos and at an early stage the gonadal development is termed *indifferent* or *ambisexual*. It was thought that development to one sexual phenotype or another occurred after migration of the primordial germ cells to the indifferent gonads. However, the development of male or female gonads, genital ducts and external genitalia is far more complicated, occurring as a result of a complex interplay between genetic expression, timing of development and the influence of sex hormones.

Early Gonadal Development (Ambisexual or Indifferent Stage)

The formation of the gonads is first indicated by the appearance of an area of thickened coelomic epithelium on the medial side of the mesonephric ridge in the fifth week (3.87 , 88 ). Elsewhere on the surface of the ridge the coelomic epithelium is one or two cells thick, but over this gonadal area it becomes many layered. The thickening rapidly extends in a longitudinal direction until it covers nearly the whole of the medial surface of the ridge. The thickened epithelium continues to proliferate, displacing the renal corpuscles of the mesonephros in a dorsolateral direction and itself forming a projection into the coelomic cavity, the *gonadal ridge*. Surface depressions form along the limits of the ridge which is thus connected to the mesonephros by an originally broad mesentery, the *mesogenitale*. In this way the mesonephric ridge becomes subdivided into a lateral part containing the mesonephric and paramesonephric ducts, which may be termed the *tubal fold*, and a medial part, termed the *gonadal fold*. The tubal fold also contains the nephric tubules and glomeruli at its base.

Up to the seventh week the ambisexual gonad possesses no sexually differentiating feature. From stage 15 the proliferating coelomic epithelium now forms a number of cellular *gonadal cords* (termed in some texts primary sex cords), separated by mesenchyme. These cords remain at the periphery of the primordium to form a cortex; more centrally a proliferation and labyrinthine cellular condensation of the mesenchyme of the mesonephros, including angiogenic

mesenchyme, constitute a medulla.

Paramesonephric Ducts

The paramesonephric (Mullerian) ducts initially develop in embryos of both sexes, but become dominant in the development of the **female** reproductive system; they are not detectable, however, until the embryo reaches a length of 10–12 mm (early sixth week). Development in the ambisexual period is followed by further details of the female duct maturation and finally brief notes of the limited male derivatives. Each commences as a linear invagination of the coelomic epithelium (the *paramesonephric groove*) on the lateral aspect of the mesonephric ridge near its cranial end, and its blind caudal end continues to grow caudally into the substance of the ridge as a solid rod of cells which acquires a lumen as it lengthens. Throughout the extent of the mesonephros it is *lateral* to the mesonephric duct which acts as a guide for it. At the caudal end of the mesonephros (which it reaches in the eighth week), the paramesonephric duct turns medially (3.89) and crosses *ventral* to the mesonephric duct to enter the *genital cord* (3.84A,B), where it bends caudally in close apposition with its fellow of the opposite side. The two ducts reach the dorsal wall of the urogenital sinus during the third month, and their blind ends produce an elevation on it termed the *Mullerian sinus tubercle* (3.89). Each duct consists, at the end of the indifferent stage, of vertical cranial and caudal parts with an intermediate horizontal region.

In the female the cranial part forms the *uterine tube*, and its original coelomic invagination remains as the pelvic opening of the tube, the fimbriae becoming defined as the cranial end of the mesonephros degenerates. The caudal vertical parts of the two ducts fuse with each other (3.87B) to form the *uterovaginal primordium*. This gives rise to the lower part of the uterus and, as it enlarges, it takes in the horizontal parts to form the fundus and most of the body of the adult uterus. A constriction between the body of the uterus and the cervix can be found at 9 weeks. The stroma of the endometrium and the uterine musculature (myometrium) are developed from the surrounding mesenchyme of the genital cord.

At about 60 mm CR length an epithelial proliferation (the *sinuvaginal bulb*) arises from the dorsal wall of the urogenital sinus in the region of the sinus tubercle, and its origin marks the site of the future hymen. Whether the epithelium involved in the proliferation is from the sinus (Bulmer 1957) or is epithelium of the mesonephric duct which has extended over the Mullerian tubercle (Vilas 1932; Meyer 1938; Forsberg 1963) is uncertain. The proliferation gradually extends cranially as a solid, anteroposteriorly flattened plate, inside the tubular mesodermal condensation of the uterovaginal primordium which will eventually become the fibromuscular *vaginal wall*. The caudal tip of the paramesonephric duct epithelium recedes until, at about the 140-mm stage, its junction with the sinus proliferation lies in the cervical canal.

Commencing from its caudal end, and gradually extending cranially through its whole extent, the solid plate formed by the sinus proliferation enlarges into a cylindrical structure; thereafter the central cells desquamate to establish the vaginal lumen. According to one view, the paramesonephric ducts do not directly contribute to the formation of the vagina (Frutiger 1969).

but it has also been suggested that mesonephric and paramesonephric ducts are both concerned (Linkevich 1969^[1]). As the upper end of the vaginal plate enlarges it grows up to embrace the cervix, and then is excavated to produce the *vaginal fornices*. The urogenital sinus undergoes relative shortening craniocaudally to form the *vestibule*, which opens on the surface through the cleft between the genital folds. The lower end of the vaginal plate grows caudally so that in 105-mm embryos the vaginal rudiment approaches the vestibule. It was thought that tissue added to the vaginal plate was pushed cephalically from the caudal end of the vaginal plate; however, Witschi (1970) suggested that the lower end of the vagina moves along the urethra to a separate opening in the vestibule. In fetuses of 162 mm the vaginal lumen is complete except at the cephalic end where the fornices are still solid; they are hollow by 170 mm. At approximately half way through gestation (180 mm) the genital canal is continuous with the exterior.

During the later months of fetal life the vaginal epithelium is enormously hypertrophied, apparently under the influence of maternal hormones, but after birth it assumes the inactive form of childhood (Fraenkel & Papanicolaou 1938^[2]).

The differing embryonic origins of the vaginal epithelium and uterine epithelium have been correlated with their dissimilar responses in adult life to stimulation with oestrogenic hormones (Zuckerman 1940^[3]).

In the male the paramesonephric duct mostly atrophies under the influence of *anti-Müllerian* hormone (AMH) (see p. 208^[4]) which is released locally by the Sertoli cells of the testis (see below); thus persisting vestigial structures are most likely cranially and caudally at the limits of the local effects of AMH. A vestige of the cranial end of the duct persists as the *appendix testis* (p. 1848^[5]). The fused caudal ends of the two ducts are connected to the wall of the urogenital sinus by a solid *utricular cord* of cells. In this position it soon merges with a proliferation of sinus epithelium, the *sinu-utricular cord*, similar to, but less extensive than, the sinus proliferation in the female. This proliferating epithelium is claimed to be an intermingling of the endoderm of the urogenital sinus with the lining epithelia of the mesonephric and paramesonephric ducts, which have extended on to the surface of the sinus tubercle. As the sinu-utricular cord grows, so the utricular cord recedes from the tubercle. In the second half of fetal life the composite cord acquires a lumen and dilates to form the *prostatic utricle*, the lining of which consists of hyperplastic stratified squamous epithelium. The sinus tubercle becomes the *colliculus seminalis* (Vilas 1933^[6]; Glenister 1962^[7]).

Primordial Germ Cells

The primordial germ cells are formed very early from the epiblast, as demonstrated at the 2-somite stage in the chick (Clawson & Domm 1969^[8]). They are large cells, in comparison with most somatic cells, being from 12 to 20 µm in diameter, and characterized by vesicular nuclei with well-defined nuclear membranes and by a tendency to retain yolk inclusions long after these have disappeared from somatic cells. (For the ultrastructure of human primordial germ cells consult Fukuda 1976^[9].) It is not yet established whether the primordial germ cells are derived from particular blastomeres during cleavage, if they constitute a clonal line from a single

blastomere or are the product of a progressive concentration of the nucleus of the fertilized ovum by unequal partition of this at successive mitoses (Bounoure 1939^[4]). Primordial germ cells spend the early stages of development within the extraembryonic tissues near the end of the primitive streak and in the connecting stalk. In this situation they are away from the inductive influences affecting the majority of the somatic cells during early development.

Primordial germ cells can be identified in human embryos in stage 11 when the number of cells is probably not more than 20–30 (Hardisty 1967^[4]). When the tail fold has formed they appear within the endoderm and the splanchnopleuric mesenchyme and epithelium of the hindgut as well as in the adjoining region of the wall of the yolk sac. By amoeboid movements and by growth displacement they migrate dorsocranially in the mesentery, passing around the dorsal angles of the coelom (medial coelomic bays) to reach the genital ridges from stage 15. It is believed that the genital ridges exert long-range effects on the migrating primordial germ cells which control their direction of migration and help support the primordial germ cell population.

In most vertebrates, mitosis in the germ cells is arrested after their early segregation to be renewed only when they reach the genital primordia. However, in mammals there is no such arrest and the cells proliferate both during and after migration to the mesonephric ridges; cells which do not complete this migration degenerate. After segregation the primordial germ cells are often termed *primary gonocytes*, which in turn divide to form secondary gonocytes. The distinction between the two generations is clear in most vertebrates, but the absence of mitotic arrest in mammals leads to a merging of the two stages.

While sexual differences in germ cell numbers occur in some vertebrate species, it is uncertain whether this represents an original difference at segregation or results from earlier and more rapid proliferation in one sex. No connection between numbers of primordial germ cells and fertility has been detected, but there is evidence that gross deficiency in number may affect individual fecundity (Hardisty 1967^[4]) (see also p. 211^[4]).

Development of the Gonads

The factors which lead to formation of either testis or ovary are presented below and on page 210^[4]. The morphological events occurring in each type of gonadal development are presented first.

Testis

The majority of studies support the hypothesis that the seminiferous tubules are formed from lines of epithelial cells derived from the proliferating coelomic epithelium (but also see below). The epithelial cords (3.87^[4]) lengthen partly by additions from the coelomic epithelium and encroach on the medulla, where they unite with the network derived from the mesenchyme which ultimately becomes the *testicular rete*. The primordial germ cells are incorporated into the cords, which later become enlarged and canalized to form the seminiferous tubules (see Fukuda

& Hedinger 1975^[5]). The cells derived from the surface of the early gonad form the *supporting cells* (of Sertoli). The *interstitial cells* of the testis are derived from mesenchyme and possibly also from coelomic epithelial cells which do not become incorporated into the tubules; they form, among other cells lines, the embryonic and fetal cells of Leydig which secrete testosterone. A later migration of mesenchyme beneath the coelomic epithelium forms the *tunica albuginea* of the testis. The cords of the rete testis, which canalize later, become connected to the glomerular capsules in the persisting part of the mesonephros. The rete cords ultimately become connected to the mesonephric duct by the five to twelve most cranial persisting mesonephric tubules and these become exceedingly convoluted and form the lobules of the head of the epididymis. The mesonephric duct, which was the primitive 'ureter' of the mesonephros, becomes the canal of the *epididymis* and the *ductus deferens* of the testis. The seminiferous tubules do not acquire lumina until the seventh month, but the tubules of the testicular rete do somewhat earlier.

Ovary

In its earliest stages, the ovary closely resembles the testis, although it is slower to differentiate its characteristically female features (3.87^[6]). Few, if any, of the gonadal cords invade the medulla, the majority remaining in the cortex, where they may be joined by a second proliferation from the epithelium overlying the gonad. In sections of the ovary in the third and subsequent months the cords appear as clusters of cells which may or may not contain primitive germ cells. These clusters are separated by fine septa of undifferentiated mesenchyme. An *ovarian rete* condenses in the medullary mesenchyme and some of its cords may form a junction with mesonephric glomeruli. The medulla subsequently regresses, and connective tissue and blood vessels from this region invade the cortex to form the stroma of the ovary. During this invasion the cortical cell clusters break into individual groups which surround the primordial germ cells, now *primary oöcytes*, which have entered the prophase of the first meiotic division. These cells were derived from a mitotic division of the primordial germ cells (*naked oögonia*). Their epithelial capsules consist of flattened *pregranulosa cells* derived from proliferations of coelomic epithelium (Gillman 1948^[7]). The ovary now has its *full complement* of primary oöcytes. The majority undergo atresia at various stages during their development, but the remainder resume development by completing the first meiotic division shortly before ovulation (see p. 123^[8]). The capsular cells at the same time enlarge and multiply to form the *stratum granulosum*, and as they do so they become surrounded by *thecal cells* which differentiate from the stroma.

Only the middle part of the gonadal ridge produces the ovary. Its cranial part is sterile and becomes the *suspensory ligament of the ovary* (infundibulopelvic fold of peritoneum). Its caudal region, also sterile, is incorporated in the *ovarian ligament*.

A study by Satoh (1991^[9]) disputes the origin of the cell lines responsible for production of the *gonadal supporting cells*, i.e. the Sertoli cells of the seminiferous tubules and the follicular cells of the ovary. After examination of serially sectioned human gonads from 5 weeks (stage 14) to 13 weeks gestational age, Satoh noted the initial proliferation of the *coelomic epithelium* which formed *primary sex cords*'. He further identified epithelial cells subsequently emerging from the distal ends of the *mesonephric tubules* where the basal lamina is absent, terming these

primordial sex cords'; they branch from the mesonephros towards the coelomic epithelium, although there is no continuity between these cells and the coelomic epithelium. He notes that the basal lamina of each primordial sex cord is contiguous with that of the mesonephros. He interprets the coelomic proliferation and production of the primary sex cords of coelomic cells as a preparation for the prominent protrusion of the gonad into the coelom which occurs rapidly, within half a day (from late week 5 to early week 6), after which the coelomic cells are arranged two or three cells deep with a basal lamina.

If the above description is upheld by other studies, the rete cords in the testis may be reinterpreted as outgrowths of the mesonephric tubules which are proliferating to produce the primordial sex cords. In ovarian development Satoh (1991^[4]) describes primordial sex cords, similar to those in the developing testis, originating from the mesonephros but notes they display an incomplete basal lamina. Later the primordial sex cords are displaced into the cortex. Satoh noted no secondary proliferation of the coelomic epithelium into the cortex of the ovary.

The extent of the mesonephric contribution to the gonads has been examined in the mouse and rabbit but as yet no clear conclusion as to the origin of the primary sex cords and Sertoli cells has been formed (Buehr et al 1993^[5]). It is anticipated that immunohistochemical techniques will reveal the origin of the cells which ultimately surround the primordial germ cells. Further confirmation of this work is awaited with interest.

Sex Determination in the Embryo

It was believed that the gonads were indifferent or ambisexual until the arrival of the primordial germ cells in the gonadal ridge, when the sex of the embryo was 'turned on' by the presence of the male or female germ cells. It now seems that the germ cells may be essentially irrelevant to *testis determination*; embryos in which the genital ridges are devoid of germ cells may still have morphologically normal testis development (McLaren 1985^[6]). It is not clear if the germ cells are necessary for ovarian determination; however, they **are** required for the proper organization and differentiation of the ovary: their absence results in the development of 'streak gonads', where only lines of follicular cells can be seen, as in Turner's syndrome (see p. 122^[7]).

The processes of sex determination and differentiation are now seen to involve interacting pathways of gene activity which lead to the total patterning of the embryo to one or other sex. In one model of determination in humans, the female pathway is considered the default pathway; the Y chromosome of a male embryo diverts development into the testicular pathway and the resultant changes of the indifferent gonad to a testis produces a range of local and widely acting hormones which generate all the secondary sexual characteristics. (For a different model see Gilbert 1991^[8].) The possession of a Y chromosome is usually associated with a male developmental pathway. The male determining region of the Y chromosome is located near its tip and termed the *testis-determining factor* (TDF). This is regarded by some workers as the 'master switch' which programmes the direction of sexual development. It is suggested that the TDF acts initially within the population of cells which will form the sex cords of the ambisexual gonad; these will differentiate into the support cells for the germ cells in both testis and ovary.

TDF alters their subsequent development away from that of the female default pathway (i.e. into follicular cells) to that of Sertoli cells (Burgoyne et al 1988; Palmer & Burgoyne 1991). The Sertoli cells then influence the differentiation of the other cell types in the testicular pathway, e.g. Leydig cells appear some time later, and the connective tissue becomes organized into a male pattern. The germ cells are also affected by this environment when they arrive: they become enclosed within the Sertoli cells and thus enter mitotic arrest which is characteristic of spermatogenesis, instead of entering meiosis and meiotic arrest which is seen in oögenesis.

Subsequent differentiation into the male line is caused by the production of two factors: Sertoli cells make AMH (also called *Mullerian inhibiting substance* or MIS), which causes the regression of the Mullerian ducts (Josso & Picard 1986); Leydig cells produce testosterone which promotes the development of the mesonephric ducts (Grumbach & Ducharme 1960), sets into process the development of male external genitalia and sensitizes other tissues to testosterone (see below—descent of the testis). Thus the development of male characteristics follows the expression of TDF, and female characteristics develop in its absence. Sex determination in mammals thus may be initiated by a signal which switches on TDF.

Further studies on the exact position of the TDF have been based on deletion mapping the Y chromosome in a class of XX males that arise from abnormal X:Y interchange at meiosis (Petit et al 1987). A conserved sequence that mapped to the Y chromosome of all mammals tested was found. The sequence formed part of a gene in the *Sex determining Region of the Y chromosome* and was thus termed SRY (see also p. 211). It is believed to be genetically and functionally equivalent to TDF. In the mouse, this gene (termed Sry) has been demonstrated, by cells in the genital ridge only, for a brief period before testis differentiation. This gene has also been seen in mutant strains which lack germ cells, indicating that the gene is expressed in a somatic cell line within the genital ridge. The potential of the Sry gene was demonstrated by injecting it into fertilized mouse eggs which were reimplanted and allowed to develop. Examination of the gonads showed testes developing in chromosomal female animals indicating that Sry alone is able to initiate male development in a chromosomally female embryo (Koopman et al 1991). Interestingly it is also suggested that there must be other genes required for the development of the male phenotype that reside on chromosomes **other** than the Y (Lovell-Badge 1992).

Although the possession of a Y chromosome expressing SRY and TDF is, in many studies, seen as the fundamental cause of the switch to male phenotypic development, by initiating Sertoli cell differentiation, other studies have suggested that the possession of TDF **accelerates** the development of the gonads in XY embryos generally, so that testes are larger and more advanced than ovaries of the same age (Mittwoch 1988).

At the time of Sertoli cell differentiation in mice, the gonads of XY fetuses are on average 40% larger than their XX littermates, suggesting that the putative testes grow faster than putative ovaries before any morphological differentiation can be seen. Bovine male embryos generally develop to more advanced stages than do females during the first 8 days after insemination in vitro, well before gonadal differentiation (Xu et al 1992). Male human fetuses are generally bigger than females from 12 weeks gestation, and males are already slightly ahead of females at

six weeks gestation just prior to testicular differentiation; it is suggested that this difference in the growth rate is encoded in the sex chromosomes (Pedersen 1980^[1]). Once gonadal development has commenced the difference in size between testes and ovaries becomes much greater than the difference between XY and XX fetuses as a whole. (Interestingly, the right gonad develops slightly ahead of the left, an observation which correlates with hermaphrodite development in which testes are more often on the right side and ovaries on the left.)

Mittwoch (1988^[2]) suggests that whereas in poikilotherms the sex of the embryo is determined by the temperature of incubation, homoiothermic animals have evolved a genetic mechanism to make sex determination independent of the environmental temperature. As mammals also have an in utero environment which receives female hormones from the mother, the accelerated development of the testis at the early embryological stages ensures arrest of meiosis of the germ cells and the production of local hormones which masculinize the male embryo before the normal time for development of the reproductive tract and ovaries of the female.

The early expression of Sry has been demonstrated in the mouse embryo where two sex-determining regions, Sry and Zfy, are transcribed during preimplantation development, as early as the two-cell stage (Zwingman et al 1993^[3]).

The range of intersex conditions, of phenotypic sex which is not correlated to genotype, and the effect of multiple X chromosomes in males suggest that there may be many testis determining genes necessary for the male developmental pathway but only a single X chromosome for the female default pathway (Mittwoch 1992^[4]). Certainly once testicular differentiation and male hormone secretion have begun, other Y-chromosomal genes are required to maintain spermatogenesis and complete spermiogenesis. The impairment of oögenesis, by other chromosomal abnormalities, is much less severe than the impairment of spermatogenesis.

The source of the X chromosome may be an important factor in this paradigm; in mice a paternally derived X chromosome has been shown to have a retarding effect on development (Thornhill & Burgoyne 1993^[5]). More work in this area is awaited with interest.

Environmental Effects on Gonadal Development

Disorders of development of the testis and reproductive tract in the male fetus are increasing in incidence. Testicular maldescent (cryptorchidism) and hypospadias appear to have doubled or trebled in incidence in the last 30–50 years, according to the best evidence (Giwerzman & Skakkebaek 1992^[6]; Sharpe & Skakkebaek 1993^[7]), whilst testicular cancer has increased by an even greater margin to become now the commonest cancer of young men (Skakkebaek et al 1993^[8]). Although testicular cancer is primarily a disease of young men (95% of cases affect 15–45-year-old males) it is now established that this age-incidence reflects activation of pre-malignant carcinoma-in-situ (CIS) cells which are present at birth and which almost certainly arise during fetal life (Skakkebaek 1987^[9]; Skakkebaek et al 1993^[10]). Current opinion is that these CIS cells are primordial germ cells which have failed to develop normally. It is of interest that

abnormalities of development of the testis and reproductive tract (e.g. gonadal dysgenesis, cryptorchidism, small testes) are important risk factors for the development of testicular cancer (Giwerzman & Skakkebaek 1992; Brown et al 1992). However, the most dramatic change that appears to have occurred over the past 50 or so years is a fall in sperm counts in man of around 40–50% (Carlsen et al 1992). Although this dramatic decrease is obviously manifest only in adulthood, as with testicular cancer, the most likely explanation is impaired testicular development during fetal or childhood life (Sharpe 1993; Sharpe & Skakkebaek 1993).

The **Sertoli cells** are one of the first specialized somatic cells of the putative testis (see above); they briefly express Sex determining Region of the Y chromosome (SRY) before testis differentiation commences. Sertoli cells proliferate during these early stages and continue to do so for some or perhaps all of subsequent childhood life (Cortes et al 1987; Sharpe 1994); when replication ceases the Sertoli cells mature and cannot be reactivated. The importance of this period of Sertoli cell proliferation lies in the fact that each Sertoli cell can only support a fixed number of germ cells during their development into spermatozoa, i.e. the number of Sertoli cells produced at this time determines the maximal limit of sperm output (Sharpe 1994). Additionally, because it is the germ cells which comprise the bulk of the adult testis, then the number of Sertoli cells also predetermines the size to which the testes will grow; however, it should be kept in mind that factors which impair the process of spermatogenesis, resulting in the loss of germ cells, will also affect testicular size.

Studies in a range of laboratory and domestic animals have shown that differences in the number of Sertoli cells is the primary determinant of differences in testicular size and sperm output between species, between strains and between individuals (Sharpe 1994). The same is true for man: variation in Sertoli cell number is probably the most important factor in accounting for the enormous variation in sperm counts between individual men, whether fertile or infertile (MacLeod & Gold 1951; Carlsen et al 1992). Indeed, the available data for adult men indicates that Sertoli cell numbers vary across a 50-fold range (Johnson et al 1984). Although some of this variation may result from attrition of Sertoli cell numbers because of ageing (Johnson et al 1984), the major differences in Sertoli cell numbers will have been determined by events in fetal and/or childhood life (Sharpe 1994).

Sharpe (1994) has raised the question as to whether the reported 50% fall in sperm counts in men over the last half-century might be due to a secular decrease in the number of Sertoli cells. There are no data on comparative Sertoli cell numbers from 50 years ago. Alterations of Sertoli cell numbers, whether up or down, would not alter the quality of the spermatozoa produced and, histologically, such testes with lower Sertoli cell numbers could not be distinguished from those with higher numbers—only their overall size would be different (Sharpe 1994). Thus, the absence of any dramatic increase in the incidence of male infertility over the last half-century would not be inconsistent with a decrease in Sertoli cell numbers over the same time period. However, other early events controlled by the Sertoli cells (testicular descent, masculinization, germ cell development) appear to be affecting an increasing proportion of human males. Comparable effects are also occurring in a range of wildlife (Colborn & Clements 1992), suggesting that environmental factors are most likely to blame. Sharpe (1994) suggests that *environmental oestrogens* may be the most likely causal agent, but warns that there is as yet

no definitive proof that this is the case.

It is well established that administration of exogenous oestrogens to pregnant animals during the period when testicular differentiation and 'masculinization' are occurring in the male fetus will lead to abnormalities in these processes, resulting in increased risk of cryptorchidism and hypospadias at birth and smaller testes and reduced sperm counts in adult life (Arai et al 1983; Greco et al 1993; Sharpe & Skakkebaek 1993). The same is true for man, based on studies of the male offspring (Whitehead & Leiter 1981; Stillman 1982) of some of the 6 million women worldwide who were administered diethylstilboestrol (DES), in the period 1945-70, in the belief that it would prevent miscarriage. The basis for these oestrogen-induced abnormalities has not been fully worked out, but it is probably significant that testicular differentiation and masculinization are very early events occurring at a time when oestrogen levels in the maternal circulation are still relatively low (Tulchinsky et al 1972; Tulchinsky & Hobel 1973).

Sharpe and Skakkebaek (1993) suggest that there has been a generally increased human exposure to oestrogens over the past 50 years. This has been caused by changes in diet and body composition (more fat), leading to increased exposure of women to their own oestrogens (Adlercreutz 1990), and increased exposure to environmental oestrogens, the most important of which are a range of ubiquitous, pollutant chemicals which, when ingested, mimic the effects of oestrogens in the body by interacting with the receptors for oestradiol. Pesticides such as DDT (and its metabolite DDE) and other widely distributed compounds such as polychlorinated biphenyls (PCBs), though their use is now largely banned, continue to accumulate in living organisms because of their fat solubility. The bioaccumulation is a feature of many chlorinated hydrocarbons, several of which appear to be oestrogenic (Hileman 1993). More recently, degradation products of non-ionic surfactants (used widely in commercial detergents) have also been shown to be oestrogenic and these again are widely distributed, bioaccumulative and are present in some water sources and food chains (Clark et al 1992; Zoller 1993). It remains unknown whether the level of human exposure to such oestrogenic chemicals is sufficient to exert adverse effects on reproductive development of the early male fetus or developing child, but the increasing prevalence of such disorders in man and wildlife provides at least circumstantial support for this possibility (Sharpe & Skakkebaek 1993). More definitive evidence should become available in the next few years.

Sharpe (1994) urges that irrespective of whether increased human exposure to oestrogens is responsible for the increasing incidence of male reproductive abnormalities, it must be noted that reproductive ability of the adult human male is, to a considerable extent, predetermined by events in fetal life and/or childhood. He perceives a fairly urgent need for increased understanding of these early processes and their vulnerability to an altered hormonal milieu, in order that their importance in determining subsequent male fertility/ infertility can be understood.

Descent of the Gonads

Descent of the Testis

This is **not** merely a simple migration. At first the testis lies on the dorsal abdominal wall, but, as it enlarges, its cranial end degenerates and the remaining organ therefore occupies a more caudal position. It is attached to the mesonephric fold by a peritoneal fold, the *mesorchium* (3.82) (the mesogenitale of the undifferentiated gonad), which contains the testicular vessels and nerves and a quantity of undifferentiated mesenchyme. In addition, it acquires a secondary attachment to the ventral abdominal wall, which has a considerable influence on its subsequent movements. At the point where the mesonephric fold bends medially to form the genital cord (p. 200), it becomes connected to the lower part of the ventral abdominal wall by an *inguinal fold* of peritoneum (3.84). The mesenchymal cells occupying the core of the inguinal fold condense as another cord, the *gubernaculum*, extending from the epidermal ectoderm which will later form the scrotum, through the inguinal fold and the mesorchium to the caudal pole of the testis. It traverses the site of the future inguinal canal, which is formed around it by the muscles of the abdominal wall as they differentiate. At the end of the second month the caudal part of the ventral abdominal wall is horizontal but, after the return of the intestine to the peritoneal cavity (p. 334), it grows in length and progressively becomes vertical. As a result, the umbilical artery pulls up a falciform peritoneal fold, as it runs ventrally from the dorsal to the ventral wall, and this forms the medial boundary of a peritoneal fossa into which the testis projects. This fossa is the *saccus vaginalis* or *lateral inguinal fossa* (p. 1737) and its lower end protrudes down the inguinal canal along the ventrosuperior aspect of the gubernaculum, as the *processus vaginalis*. The caudal pole of the testis is *retained* in apposition with the deep inguinal ring by the gubernaculum until the seventh month, when it abruptly and rapidly passes through the inguinal canal and gains the scrotum. As it descends it is necessarily accompanied by its peritoneal covering, and the adjoining peritoneum from the iliac fossa is drawn down into the processus vaginalis. The distal end of the processus vaginalis, into which the testis projects, forms the *tunica vaginalis testis* but the portion associated with the spermatic cord in the scrotum and in the inguinal canal normally becomes obliterated, usually leaving a fibrous remnant. Sometimes the remnant atrophies completely. Alternatively, its original cavity may persist in whole or in part and in any location. These variations may form the walls of hernial sacs or encysted fluid sites.

The mechanism of the descent of the testis has variously been ascribed, by different investigators, to shortening and active contraction of the gubernaculum, to increased intra-abdominal pressure, to a simple growth process and to the effect on the convex surface of the gland of the active contraction of the lower fibres of the internal oblique muscle, squeezing it through the canal. The gubernaculum precedes the testis both spatially and in rate of growth, forming a tapering column of soft tissue with the diminutive testis at its cranial pole. It continues to grow until the seventh month, by which time its caudal part has filled the future inguinal canal and has begun to expand the developing scrotum. In this it also precedes the processus vaginalis, but does not develop attachments to skin; nor is there any evidence that it produces the radiating extensions into the suprapubic, perineal and femoral sites, which are often used as explanations for the various forms of ectopia testis. By its soft consistency the gubernacular tissue (which in the early stage is formed mainly of hyaluronic acid) may offer a route of low resistance to the descending testis, and the cessation of its growth in the last two months of gestation, coupled with an accelerating rate of growth in the testis and epididymis, may also be a factor in testicular

descent as far as the inguinal canal.

The mechanism of final, rapid descent into the scrotum is not yet clear. Endocrine effects seem certain, but this does not explain the actual agency. This account is based principally upon events as observed in porcine material by Backhouse and Butler (1960^[1]). A subsequent discussion of the problems of testicular descent and maldescent by Backhouse (1964^[2]) should be consulted. He had reviewed the literature since Hunter's original description (1762). Apparently the cremaster muscle develops in gubernacular mesenchyme and this may explain the development of the concept of the gubernaculum as a 'fibromuscular' ligament.

In the rat it has been shown that the gubernaculum is highly contractile during testicular descent. In the inguinoscrotal phase of descent the gubernaculum loses its hyaluronic acid, the cremasteric muscle develops and the processus vaginalis elongates. These processes are dependent on androgens, but attempts to isolate androgen receptors on the gubernaculum have, as yet, been unsuccessful. Division of the genitofemoral nerve, however, prevents both the inguinoscrotal testicular descent and differentiation and migration of the gubernaculum, suggesting that androgens acting on the nerve cell bodies of the genitofemoral nerve in the spinal cord could cause release of neurotransmitters from the nerve endings that might act as second messengers for androgens (Beasley & Hutson 1988^[3]). A peptide neurotransmitter, *calcitonin gene-related peptide* (CGRP), is present in the genitofemoral nerve and its cell bodies in the spinal cord. CGRP causes the gubernacula from newborn male mice to contract rhythmically; CGRP antagonists inhibit this contraction (Park & Hutson 1991^[4]). It is suggested that one of the effects of testosterone during gestation is to 'masculinize' the genitofemoral nerve by increasing the number of cells contributing to it. One cause of non-descent of the testes may be a result of insufficient testosterone during development resulting in a failure to produce enough nerve cells in the genitofemoral nerve; then, at the time of testicular migration, too little CGRP is produced to stimulate contractions in the gubernaculum and assist testicular descent.

Abnormalities of Testicular Descent

The testis may remain in the abdomen, or it may fail to reach the scrotum and may then lie in any of the following situations:


- in the perineum
- at the root of the penis
- at the superficial inguinal ring (p. 1855^[5])
- in the upper part of the thigh.


These malpositions have been traditionally associated with certain additional extensions of gubernacular tissue. The largest extension normally passes to the scrotum while lesser extensions have been described as gaining attachment to the perineum, the root of the penis, the pubis, the inguinal ligament and the neighbourhood of the saphenous opening. The testis must follow the processus vaginalis and, should the latter for any reason follow any but the scrotal extension of

the gubernaculum, malposition of the testis will result. It should be appreciated, however, that considerable doubt has now been expressed concerning these lesser expansions (previously the so-called 'tails of Lockwood'): possibly they reflect premature and abnormal fibrous partitioning of the gubernacular mesenchyme.

As noted, the processus vaginalis may remain completely patent, or its obliteration may be incomplete. When it retains a connection with the general peritoneal cavity it provides a preformed sac for a potential oblique inguinal hernia. It may be occluded at its upper end and may be shut off from the tunica vaginalis and yet remain patent in the intervening section. The patent portion may become distended with fluid, an *encysted hydrocoele* of the spermatic cord.





Descent of the Ovary


This is less extensive than the testis. Like the testis, the ovary ultimately reaches a lower level than it occupies in the early months of fetal life but it does not leave the pelvis to enter the inguinal canal, except in certain anomalies. Connected to the medial aspect of the mesonephric fold by the *mesovarium* (homologous with the mesorchium), the ovary is also attached to the ventral abdominal wall through the medium of the inguinal fold. In this fold a mesenchymatous gubernaculum also develops but, as it traverses the mesonephric fold, it acquires an additional attachment to the lateral margin of the uterus near the entrance of the uterine tube. Its lower part, caudal to this uterine attachment, becomes the *round ligament of the uterus* and the part cranial to this the *ovarian ligament*, these structures together being homologous with the gubernaculum testis in the male. This new uterine attachment may be correlated with the restricted ovarian descent. At first the ovary is attached to the medial side of the mesonephric fold but, in accordance with the manner in which the two mesonephric folds form the genital cord (p. 200 ), its connection is finally to the posterior layer of the broad ligament of the uterus. The gubernaculum thus persists in the female, unlike the male, as **two** fibrous bands or ligaments on each side. The gubernaculum ovarii does not contract as a response to CGRP (see above).


The *saccus vaginalis* also appears in the female; its prolongation into the inguinal canal (sometimes termed the *canal of Nuck*) normally undergoes complete obliteration, but may remain patent and form the sac of a potential oblique inguinal hernia (p. 1788 ). At birth the ovary and the lateral end of the corresponding uterine tube lie above the pelvic brim, and they do not sink into the lesser pelvis until the latter enlarges sufficiently to contain both of them and the other pelvic viscera, including the bladder.


Cloaca and External Genitalia

Urinary Bladder

The urinary bladder (**3.86A-F** ) is derived partly from the so-called endodermal cloaca and partly from the caudal ends of the mesonephric ducts (**3.83** , **3.86A-F** , **3.89** ). The walls of the cloaca are composed of an endodermal lining encased in intermediate mesenchyme. In


contrast the mesonephric ducts are derived from an epithelium, from the intermediate mesenchyme continuous with the coelomic epithelium, encased in intermediate mesenchyme. After the separation of the rectum from the cloaca (p. 191 ) , the ventral part of the cloaca becomes divided into three regions:

- a cranial *vesico-urethral canal*, continuous with the allantoic duct, into which the mesonephric ducts open
- a middle, narrow channel, the *pelvic* portion
- a caudal, deep, *phallic* section, closed externally by the urogenital membrane (3.86A-E ) .

The second and third parts together constitute the *urogenital sinus*. The ureter and the mesonephric duct come to open separately into the vesico-urethral part. The termination of the mesonephric duct then moves caudally to open into that part which will form the prostatic urethra. This occurs by the formation of a caudally directed loop of the duct behind the urogenital sinus, followed by absorption of the apposed walls. In this way the mesonephric duct contributes to the *trigone* of the bladder and dorsal wall of the proximal (superior) half of the *prostatic urethra*, i.e. as far as the opening of the prostatic utricle and ejaculatory ducts (or its homologue the whole female urethral dorsal wall). The remainder of the vesico-urethral part forms the body of the bladder and urethra; its apex is prolonged to the umbilicus as a narrow canal, the *urachus*. In postnatal life the urachus is drawn downwards as the bladder descends but its upper end remains connected to one or both of the obliterated umbilical arteries. Its lumen persists throughout life and its lower end frequently communicates with the bladder near its apex (Begg 1930 ) .

Cloacal Malformations

These are much more variable in their anatomic form than other congenital malformations. Two varieties can be distinguished:

1. In *extroversion of the bladder (ectopia vesicae)* the lower part of the anterior abdominal wall is occupied by an irregularly oval area, covered with mucous membrane, on which the two ureters open (Wyburn 1937 ) . Around its periphery this extroverted area, covered by urothelium, becomes continuous with the skin. This maldevelopment occurs after the separation of the ventral from the dorsal part of the cloaca. The urogenital membrane extends further cranially than it does in normal cases and the genital tubercle forms at its caudal limit. Rupture of the membrane thus throws the bladder open to the exterior.
2. In *extroversion of the cloaca* the condition is very similar, but is complicated by the presence of intestinal openings in the median plane. The urogenital sinus may remain with a high confluence of bladder, vagina and rectum. The cloacal membrane may be abnormally elongated and prematurely ruptured throughout its whole extent, prior to the formation of the urorectal septum, or, in some cases there may be only a small sinus opening externally at the skin. The anal musculature is often present but not

associated with the anal canal. (For a comprehensive discussion of cloacal malformations and cloacal exstrophy see Ricketts et al 1991¹; Hendren 1992².)

Urethra

In the male the prostatic urethra proximal to the orifice of the prostatic utricle is derived from the vesico-urethral part of the cloaca and the incorporated caudal ends of the mesonephric ducts. The remainder of the prostatic part, the membranous part and probably the part within the bulb are all derived from the urogenital sinus. The succeeding section, as far as the glans, is formed by the fusion of the genital folds, while the section within the glans is formed from ectoderm (see below).

In the female the urethra is derived entirely from the vesico-urethral region of the cloaca (see above), including the dorsal region derived from the mesonephric ducts. It is homologous with the part of the prostatic urethra proximal to the orifices of the prostatic utricle and the ejaculatory ducts.

Urethral Sphincter

This first forms as a mesenchymal condensation around the urethra in 12–15 mm (stage 18) embryos, after division of the cloaca. The mesenchyme proliferates becoming defined at the bladder neck in 31-mm embryos and along the anterior part of the urethra by 69 mm. The muscle fibres differentiate after 15 weeks gestation when both smooth and striated fibres can be seen. In females there is continuity between the smooth muscle of the urethral wall and of the bladder. In the male the muscle fibres are less abundant because of the local development of the prostate. Striated muscle fibres form around the smooth muscle initially in the anterior wall of the urethra and later they encircle the smooth muscle layer. The origin of the striated muscle is not known but could derive from the myogenic cells producing the puborectalis muscle. The smooth and striated components of the urethral sphincter are closely related but there is no mixing of fibres as seen in the anorectal sphincter (Bourdelat et al 1992³).

Defects of the Urethra

Defects due to arrests of development are not uncommon in the male. The urethra may open on the ventral (perineal) aspect of the penis at the base of the glans (see below), and the part of the urethra which is normally within the glans is absent. This constitutes the simplest form of *hypospadias*. In more severe cases the genital folds fail to fuse, and the urethra opens on the ventral aspect of a malformed penis just in front of the scrotum. A still greater degree of this malformation is accompanied by failure of the genital swellings to unite with each other. In these cases the scrotum is divided and, since the testes are also frequently undescended, the resemblance to the labia majora is very striking. Male children suffering from this deformity are often mistaken for girls.

In epispadias the urethra opens on the dorsal aspect of the penis at its junction with the anterior

abdominal wall. No satisfactory explanation has yet been suggested for this anomaly. For a genetic and epidemiological study of urinary tract malformations consult Bois et al (1975📖).

Prostate Gland

The prostate arises during the third month from interactions between the urogenital sinus mesenchyme and the endoderm of the proximal part of the urethra. (In recombinant experiments in the rat, the sinus mesenchyme is capable of inducing glandular epithelium from adult bladder.) The earlier outgrowths, some 14–20 in number, arise from the endoderm around the whole circumference of the tube, but mainly on its lateral aspects and excluding the dorsal wall above the utricular plate. These outgrowths give rise to the *outer glandular zone* of the prostate (p. 1859📖). Later outgrowths from the dorsal wall above the mesonephric ducts arise from the epithelium of mixed urogenital, mesonephric and possibly paramesonephric origin covering the cranial end of the sinus tubercle. These produce the *internal zone* of glandular tissue. The outgrowths, which are at first solid, branch, become tubular and invade the surrounding mesenchyme, which is differentiating into non-striated muscle, associated blood and lymphatic vessels and connective tissues. The mesenchyme is invaded by autonomic nerves.



Similar outgrowths occur in the female but remain rudimentary. The urethral glands correspond to the mucosal glands around the upper part of the prostatic urethra and the para-urethral glands to the true prostatic glands of the external zone (Glenister 1962📖).

The *bulbo-urethral glands* in the male, and *greater vestibular glands* in the female, arise as diverticula from the epithelial lining of the urogenital sinus.

External Genitalia

The external genital organs, like the gonads, pass through an indifferent state before distinguishing sexual characters appear (3.90👁️). Patterning of the external genitalia may be achieved by mechanisms similar to those patterning the face and limb (see p. 294📖). Homologies of the parts of the urogenital septum are shown in Table 3.1📖. From stage 13, *external genitalia primordia*, composed of underlying proliferating mesenchyme covered with ectoderm, arise around the cloacal membrane, between the primitive umbilical cord and the tail. During stage 15 the cloacal membrane is divided by the *urorectal septum* into a cranial *urogenital membrane* and a caudal *anal membrane*. Local ectodermal/mesenchymal interactions give rise to the anal sphincter which will develop without the presence of the urorectal septum or the anal canal. A surface elevation, the *genital tubercle*, appears at the cranial end of the urogenital membrane and two lateral ridges, the *genital or urethral folds*, form each side of the membrane. A distinct primordium which will become the *glans* of the penis or the *clitoris* can be recognized at the distal end of the genital tubercle. Elongation of the genital tubercle, urogenital membrane and the genital folds produces a *primitive phallus* (p. 180📖). As this structure grows it is described as having a cranial surface (analogous to the dorsum of the penis) and a caudal surface analogous to the perineal surface of both sexes. The urogenital sinus, contiguous with the internal aspect of the urogenital membrane, becomes attenuated within the elongating phallus

forming the *primitive urethra*. The urogenital membrane breaks down at about stage 19 (20 mm, 6.5 weeks) allowing communication of ectoderm and endoderm at the edges of the disrupted membrane and continuity of the urogenital sinus with the amniotic cavity. Urine can escape from the urinary tract from this time. The endodermal layer of the attenuated distal portion of the urogenital sinus now displayed on the caudal aspect of the phallus is termed the *urethral plate*. With proliferation of mesenchyme within the genital folds, the urethral plate sinks into the body of the phallus forming a *primary urethral groove*. The genital folds meet proximally in a transverse ridge immediately ventral to the anal membrane.

While these changes are in progress two *labioscrotal (genital) swellings* appear, one on each side of the base of the phallus; these extend caudally, separated from the genital folds by distinct grooves (3.90 , 91 ).

Male Genitalia

The growth of male external characteristics is stimulated by androgens regardless of the genetic sex. The male phallus enlarges to form the penis, its apex being the glans. The genital swellings meet each other ventral to the anus and unite to form the scrotum. The genital folds fuse with each other from behind forwards enclosing the phallic part of the urogenital sinus behind to form the bulb of the urethra; similarly, the folds close the definitive urethral groove in front to form the greater part of the spongiöse urethra. Fusion of the folds results in the formation of a median raphe and occurs in such a way that the lining of the postglandular urethra is mainly, perhaps wholly, *endodermal* in origin (Glenister 1954^[4]). Thus, as the phallus lengthens, the urogenital orifice is carried onwards until it reaches the base of the glans. From the tip of the phallus an ingrowth of surface *ectoderm* occurs within the glans to meet the penile urethra with which it fuses. Canalization of the ectoderm gives rise to a continuation of the urethra within the glans.

The glans and shaft of the penis are recognizable by the third month. The prepuce also begins to develop in the third month, when the urethra still has its primary external orifice at the base of the glans. A ridge consisting of a mesenchymal core covered by epithelium appears proximal to the neck of the penis and extends forwards over the glans. Deep to this ridge is a solid lamella of epithelium which extends backwards to the base of the glans. The ventral extremities of the ridge curve backwards to become continuous with the genital folds at the margins of the urethral orifice. As the urethral folds meet to form the terminal part of the urethra, the ventral horns of the ridge fuse to form the frenulum. Over the dorsum and sides of the glans, the epithelial lamella breaks down to form the preputial sac and thus free the prepuce from the surface of the glans. There-after the prepuce grows as a free fold of skin covering the terminal part of the glans. The preputial sac may not be complete until 6–12 months or more **after birth** and, even then, the presence of some connecting strands may still interfere with the retractability of the prepuce.


The mesenchymal core of the phallus is comparatively undifferentiated in the first 2 months, but during the third month the blastemata of the corpora cavernosa become defined. Nerves are present in the differentiating mesenchyme from the seventh week (Dail & Evan 1974^[4]).

Female Genitalia

The female phallus, which exceeds the male in length in the early stages, becomes the clitoris. The genital swellings remain separate as the labia majora and the genital folds also remain separate, forming the labia minora. The perineal orifice of the urogenital sinus is retained as the cleft between the labia minora, above which the urethra and vagina open. The prepuce of the clitoris develops in the same way as its male homologue. By the fourth month the female external genitalia can no longer be masculinized by androgens.

Hormonal Control of Genital Development

Development of the male phenotype requires fetal secretion of both testosterone and AMH, and development of the appropriate cytoplasmic testosterone-binding protein. Absence of the testosterone-binding protein results in XY individuals with testes and degenerated Mullerian ducts, but because they cannot respond to the circulating testosterone produced by their testes they develop female secondary sexual characteristics.

There is evidence that in certain tissues testosterone is converted into 5α -dihydrotestosterone, for example urogenital sinus and genital swellings. In XY individuals with a genetic deficiency of the enzyme responsible for this conversion, functioning testes are present but also female external genitalia with an enlarged clitoris and a small vaginal pouch, suggesting that external genital development is under the control of 5α -dihydrotestosterone. Such individuals are usually raised as girls. However, at puberty the external genitalia become responsive to testosterone causing masculinization at this stage (Imperato-McGinley et al 1974.



It is apparent that the hormones produced by the fetal gonads act on targets other than internal and external genitalia; for example, the number of cell bodies contributing to the genitofemoral nerve is higher in males (see above), thus promoting descent of the testis. Sexual dimorphism has been noted in the brain of many species. The ability to detect testosterone receptors may elucidate many more sites of testosterone sensitivity than has been previously supposed.





Nervous System and Special Sense Organs

The nervous system is divided into the *central nervous system* (CNS), which includes the brain and spinal cord, and the *peripheral nervous system* (PNS), which includes neuronal cell bodies outside the CNS and the nerves which take information to and from the brain and spinal cord. At a functional level those nerves which carry conscious sensations and innervate striated muscle (derived from axial and paraxial mesenchyme) are termed part of the *somatic nervous system*, whilst control of smooth muscle (derived from splanchnopleuric mesenchyme) resides with the *autonomic nervous system*, which is subdivided into *sympathetic* and *para-sympathetic moieties*.

The entire nervous system and the special sense organs originate from three sources each derived

in turn from specific regions of the early epiblast generally termed neural ectoderm. The first source to be delineated is the *neural plate* which forms the CNS, the *somatic motor nerves* and the *preganglionic autonomic nerves*. The second source is from cells at the perimeter of the neural plate which remove themselves by epithelial/mesenchymal transition from the plate just prior to its fusion into a neural tube; these are the *neural crest cells* which form nearly all of the PNS, including the *somatic sensory nerves*, the *somatic* and *autonomic ganglia*, *postganglionic autonomic nerves* and *adrenal* and *chromaffin cells*; they also give rise to significant mesenchymal populations in the head. The third source is from *ectodermal placodes*; these are groups of cells which originate at the edge of the neural plate but remain in the surface ectoderm after neural tube formation undergoing epithelial/mesenchymal transition after the neural crest cells have commenced their migration. Ectodermal placodes contribute to the *somatic sensory ganglia* of the cranial nerves, to the *hypophysis*, the *inner ear* and, by a non-neuronal contribution, to the lens of the eye.

With the initiation of gastrulation, the first populations of epiblast cells to invaginate through the primitive streak form the prechordal plate, embryonic endoderm and notochord (see p. 144). These cells invaginate through the rostral end of the primitive streak (Hensen's node) and form a midline strip (chordamesoderm) subjacent to the overlying epiblast. Other epiblast cells passing through the lateral regions of Hensen's node, and the middle and caudal regions of the primitive streak, migrate laterally between the epiblast and the spreading embryonic endoderm and give rise to discrete populations of intraembryonic mesoblast. Those cells arising from Hensen's node and the rostral regions of the primitive streak remain close to the notochord as the paraxial mesenchyme, which later segments to form the somites; cells arising from the middle of the primitive streak migrate laterally, rostrally and caudally towards the edges of the embryonic disc and form the lateral plate mesenchyme (see p. 155). The epiblast cells remaining after gastrulation are designated surface ectoderm above the lateral plate mesenchyme and neurectoderm (neural plate) medially above the notochord and paraxial mesenchyme. Rostral to the notochord, prechordal mesenchyme forms a continuous mesenchymal network underlying the neurectoderm to which it is closely apposed.

The neural plate is a thickened epithelium, roughly oval but wider rostrally and narrowed caudally (3.46). The lateral edges of the plate become elevated as *neural folds* and approach one another to fuse in the dorsal midline as the *neural tube*. The early neural tube is coextensive with the notochord, stretching from the future cloacal membrane to the buccopharyngeal membrane. Studies on amphibian and avian embryos have suggested that Hensen's node gives rise not only to notochordal cells but also to the overlying midline region of the neural plate which, after neurulation, will become the floor plate of the neural tube (Jessell et al 1989). The node does not, however, give rise to other regions of the neural plate. The notochord is important for the maintenance and later development of the floor plate of the neural tube (p. 226); axial extension of the floor plate in vitro requires the presence of underlying notochordal cells (Keller 1985). Extirpation of notochordal cells in amphibia prior to neural tube closure results in the absence of a floor plate, whereas removal of the notochord at a later stage does not affect floor plate development.



Despite the profound developmental modifications introduced by the secondary reduction of

yolk in mammalian secondary oöcytes associated with viviparity (p. 95¹), it is widely assumed that similar *primary mechanisms* operate throughout the Chordata, including mankind. Certainly the requirement for cell interactions during early development, including neural induction, is conserved throughout many chordate groups. (For general reviews of neural induction consult Papalopulu & Kintner 1994²; Ruiz i Altaba 1993³).













Neural Tube


The physical process of *neurulation* occurs well after the onset of neural induction. The edges of the neural plate, which are continuous laterally with the surface ectoderm, roll up and fuse in the dorsal midline approximately at the cervical level (4th somite; 3.92⁴). Fusion then proceeds rostrally and caudally. Formation of the neural tube by elevation and fusion of the neural folds is termed *primary neurulation*. *Secondary neurulation* occurs in the lumbosacral region of the spinal cord in birds and mammals, and involves cavitation of a compact mass of cells. When the neural tube is closing, its walls consist of a single layer of columnar neural epithelial cells, the extremities of which abut on *internal* and *external limiting membranes*. The mechanism of rounding up of the neural plate into a neural tube has been studied particularly closely in amphibia (Burnside 1971⁵). The columnar cells increase in length and develop numerous longitudinally disposed microtubules, whilst the borders of their luminal ends are firmly attached to adjacent cells by junctional complexes; the cytoplasmic aspect of the complexes being associated with a dense paraluminal web of microfilaments (see p. 28⁶; also Watterson 1965⁷). It is proposed that this disposition of organelles imparts a slight wedge conformation on at least some of the cells. In addition, nuclei assume basal positions that enhance cell wedging in two lateral and one medial 'hinge' regions, the latter being the floor plate (Schoenwolf & Smith, 1990⁸). Together these factors result in neural groove and eventually neural tube formation. Soon, however, some of the peripheral cytoplasmic processes become detached from the (basal) external limiting membrane and rounded cells appear close to the inner membrane which, by their repeated mitotic division, form descendants which migrate outwards to take up an intermediate position in the wall of the tube. Histologically at this stage, therefore, the wall of the tube presents three zones or layers (3.93⁹, 94¹⁰, 95¹¹). The *internal ventricular zone* (variously termed the *germinal*, *primitive ependymal* or *matrix layer*) consists of the nucleated parts of the columnar cells and the round cells undergoing mitosis. The *mantle zone* (also termed *intermediate zone*) consists of the migrant cells from the divisions occurring in the deeper layer just described. The outer *marginal zone*, for a period, consists of the external cytoplasmic processes of some of the original columnar cells, but it is soon invaded by tracts of axonal processes which grow from neuroblasts developing in the mantle zone, together with varieties of non-nervous cells (glial cells and later vascular endothelium and perivascular mesenchyme).

Prior to the closure of the neural tube the neural folds become expanded considerably in the head region as a first indication of a brain. Subsequent to the closure of the rostral neuropore (p. 146¹²) these regional expansions form the three *primary cerebral vesicles* (3.96A-C¹³). The term 'vesicle' may be rather a misnomer as it suggests an exaggerated view of these localized accelerations of growth in the wall of the brain (O'Rahilly & Gardner 1971¹⁴). The bulging is not initially marked, and the vesicles are more likely gently fusiform tubes. The three regions are

named (rostrocaudally) the *prosencephalon* or *forebrain*, the *mesencephalon* or *midbrain*, and the *rhombencephalon* or *hindbrain*, the latter being continuous caudally with the spinal cord. As a result of unequal growth of its different regions three flexures appear in the brain; two of these are concave ventrally and there are corresponding flexures of the head. The first of these flexures is associated with the formation of the head fold and forms ventral to the midbrain. Due to this *mesencephalic flexure* the forebrain bends in a ventral direction around the cephalic end of the notochord and foregut until its floor lies almost parallel with that of the hindbrain (3.96 ). The second bend appears at the junction of the hindbrain and spinal cord, the *cervical flexure* (3.96 ). This increases from the fifth to the end of the seventh week, by which time the hindbrain forms nearly a right angle with the spinal cord; after the seventh week, however, extension of the head takes place and the cervical flexure diminishes and eventually disappears. The third bend, the *pontine flexure*, is at the level of the future pons. It differs from the other two in that its convexity is directed ventrally and it does not substantially affect the outline of the head.

Concomitant with the development of the flexures, the regions of the brain enlarge allowing subdivisions of some of the original vesicles. The prosencephalon can be subdivided into two parts: the *telencephalon*, lateral evaginations of the early prosencephalon which will give rise to the cerebral hemispheres, and the *diencephalon*, the remaining midline portion of the prosencephalon. The mesencephalon remains undivided. The rhombencephalon is subdivided by the pontine flexure into the *metencephalon* rostrally which will give rise to the future pons and cerebellum and the *myelencephalon* caudally which will become the future medulla oblongata.

In addition to these gross divisions, the neural tube manifests a number of ridges and depressions which subdivide it further. Prominent among these are the serial bulges that appear very early in the rhombencephalon, before the main flexures of the neural tube develop. These bulges are termed *rhombomeres*, and apparently constitute the primary units of patterning in this region (see p. 125 ). Although bulges have also been observed in other brain regions, their developmental significance is only starting to be evaluated. For more recent accounts of the early development of the brain consult Bartelmez & Dekaban (1962 ), Jacobson (1970 ), Gaze (1970 ), Eccles (1973 ), Gottlieb (1973 ), Mark (1974 ), Rakic (1981 , 1982 ), Smart (1982 , 1983 ) , Schoenwolf and Smith (1990 ).

During neurulation the neural tube becomes subdivided in the dorsoventral axis. In the ventral midline lies the *floor plate* (see 3.93 ), a region containing non-neuronal cells that plays a role in patterning the dorsoventral axis, induces motor neurons, and, later, serves as a site for ventral commissures of nerve fibres. As the neural tube closes, cells arising from the edges of the neural folds form a distinct, transitory population in the dorsal midline outside the neural tube; this is the *neural crest*. This cell population lies between the neural tube and the surface ectoderm prior to migration to diverse locations throughout the embryo. Neural crest derivatives include the sensory ganglia of the cranial and spinal nerves, the autonomic ganglia and nerves, the enteric nervous system, Schwann cells, pigment cells, odontoblasts, meninges and ectomesenchyme cells of the pharyngeal arches. The departure of the neural crest is followed by the formation of another specialized non-neuronal region dorsally, the *roof plate*, which, in some regions, forms dorsal commissures.

At first the neural tube caudal to the brain is oval in outline and its lumen is narrow and slit-like (3.93). As the lateral walls thicken, the lumen, now the central canal, widens in its dorsal part and is somewhat diamond-shaped on cross-section (3.93). The widening of the canal is associated with the development of a longitudinal *sulcus limitans* on each side. This divides the ventricular and mantle (intermediate) zones in each lateral wall into a *ventrolateral* or *basal lamina* and a *dorsolateral* or *alar lamina*. This separation indicates a fundamental functional difference. Within the spinal cord, the basal plate is concerned with motor function, containing the cell bodies of motor neurons of the anterior and lateral grey columns, while the alar plate receives sensory inflow from the dorsal root ganglia. Motor and sensory axons combine to form the segmentally arranged spinal nerves. In the head, the cranial nerves form a continuation to the series of spinal nerves, but are functionally and anatomically specialized. An important developmental contribution to the nervous system is also made by *neurogenic* (ectodermal) *placodes*, which are thickened regions of ectoderm in the head (see p. 257).

The long held and widely used terms:

- roof plate
- floor plate
- alar lamina
- basal lamina

were changed to:

- dorsal lamina
- ventral lamina
- dorsolateral lamina
- ventrolateral lamina

in the *Nomina Embryologica* associated with the *Nomina Anatomica* (fourth edition) by the International Anatomical Nomenclature Committee (Tokyo 1975). The modified names are more apposite **throughout** the *early* neural tube (i.e. spinal cord and brainstem); however, these terms become much less appropriate in the rostral half of the medulla oblongata and caudal half of the pons, with the profound positional changes accompanying the formation of the pontine flexure—3.96A. The older terminology is still retained by some research groups.)

Failure of neurulation produces the conditions of *craniorachischisis*, where the entire neural tube is unfused in the dorsal midline, *cranioschisis* or *anencephaly*, where the neural tube is fused dorsally to form the spinal cord but is not fused dorsally in the brain, and *spina bifida*, where local regions of the spinal cord, meninges and vertebrae may be malformed (p. 340, 3.197). Anencephalic fetuses have severe disturbances in the shape, position and ossification of the

basichondrocranium and in the course of the intracranial notochord.

Neural Crest

The neural crest is a **transient** structure found only in *vertebrate embryos*. It is derived from the lateral ridges of the neural plate (also called neural folds) and its appearance follows a craniocaudal gradient as the neural tube closes on the mediodorsal line. The neural crest in the chick was first described in 1868 by Wilhelm His who called it *Zwischenstrand*, to emphasize its intermediate position between the neural tube and surface ectoderm. Wilhelm His also recognized that the neural crest was the origin of the spinal ganglia—hence the term *ganglion crest* that has also been used to designate this structure. The fact that the neural crest yields mesenchymal cells was first proposed at the turn of the century by Katschenko (1888^[1]), Goronowitsch (1892^[2]) and later by Platt (1893^[3]) who showed that it contributes to the cartilage of pharyngeal arches and to the dentine of the teeth in lower vertebrates. Platt coined the term *mesectoderm* to designate the mesenchyme of ectodermal origin, a notion that challenged the current germ layer theory of von Baer (1828^[4]) (see p. 92^[5]).

Since these early times, the neural crest has attracted much attention from embryologists who, during the first half of this century, investigated the fate of this structure mainly in the amphibian embryo. The fact that neural crest cells migrate and settle in elected sites in the embryo, where they differentiate into a large variety of cell types, was deduced from ablation experiments, cell marking studies that involved the use of vital dyes, and experiments that relied on differences in cell size or pigmentation between related species of amphibians. However, none of these methods provided stable and specific markers so the migratory cells could only be distinguished for a short period of time. Nevertheless, the pluripotentiality of the neural crest was recognized and its role in contributing both to the PNS and to the facial skeleton was established.

A more precise cell marking technique was devised later by Weston (1963^[6]) and Chibon (1964) who labelled the DNA of migrating neural crest cells with tritiated thymidine (³H-TdR). Owing to the rapid proliferation of embryonic cells the label is soon diluted so that it is not stable, and, furthermore, re-uptake of label released by dead cells compromised specificity. More recently methods based on the use of liposoluble carbocyanide dyes (diI, diO) have been used to label cell membranes in living cells. While easy to use and providing conspicuous labelling these methods also have the disadvantage of being neither precise nor stable.

Quail-Chick Chimeras


From the late sixties, up to recent years, most investigations on the neural crest have used the avian embryo. The introduction of the quail–chick marker system to neural crest embryology by Le Douarin (1969^[7] et seq) has allowed the migration and fate of neural crest cells to be followed precisely up to the stage where they become fully differentiated. This technique is based on an observation by Le Douarin (1969^[8]), on the structure of the interphase nucleus in the Japanese quail. The cells of this species are characterized by the condensation of constitutive



heterochromatin into a large mass, generally centronuclear, associated with the nucleolus. Although some variations in the distribution of heterochromatin and nucleolar RNA exist in the different cell types of the quail, this mass of heterochromatin is present in all embryonic and adult cell types of this species (Le Douarin 1973📖). Such a characteristic is rare in the animal kingdom; in most species the constitutive heterochromatin is evenly distributed in the nucleoplasm in small chromocentres as it is, for example, in the chick (3.97👁️). Quail and chick cells can thus easily be distinguished by DNA staining or by electron microscopy where the large, DNA-rich nucleolus of the quail is easily recognizable. These species–species differences have been used to study the migration of embryonic cells and to analyse the contribution of cells of different embryonic origins to complex tissues or organs during ontogeny. Since the quail and the chick are closely related in taxonomy, the substitution of definite territories between embryos of these two species in ovo results in viable *chimeras* which develop normally and can hatch. Thus, when definite fragments of either the neural fold or the neural tube and associated neural crest of the quail are grafted *isotopically* or *isochronically* into the chick (or vice versa) the migration and fate of their constituent cells can be followed at any time after the operation (3.98👁️). This approach has been systematically applied to the whole neuraxis to establish the fate map of the neural fold and to define the paths along which neural crest cells migrate. In addition to the nuclear marker, species specific antibodies and cDNA probes have been used to analyse the chimeras (Le Douarin 1993📖; Izipisua-Belmonte et al 1993📖).



Fate of the Head Neural Fold



The neural fold establishes a **transition** between the neural ectoderm, i.e. the neural plate which becomes the brain and spinal cord, and the surface ectoderm which differentiates later into epidermis. Along most of the neuraxis, the cells forming the neural fold undergo an epithelial/mesenchymal transformation through which they acquire the migratory properties of neural crest cells. The *rostral prosencephalic neural fold*, however, does **not** generate neural crest. Quail-chick chimera recombination in embryos at the neurula stage (0-3 somites) (3.99👁️), demonstrated that neural crest cells are produced along the neural axis rostrally as far as the diencephalon, at about the level from which the epiphysis arises. The rostral neural fold itself gives rise to the *hypophyseal placode*, i.e. the future Rathke's pouch, the *olfactory placodes* and associated *nasal epithelium*, including the roof of the nasopharynx and, more caudally, the skin of the upper lip (beak in chick) and the frontonasal area. Extrapolation to the human and the results obtained in quail–chick chimeras is represented in 3.100👁️. Studies of the fate of the neural fold caudal to the mid-diencephalon revealed that the *frontal* and *parietal bones* are entirely of neural crest origin (Couly et al 1992📖) (see p. 274📖) as well as the *facial* and *hypobranchial skeleton* and part of the *optic* and *otic capsules* (Le Douarin 1982📖) (see p. 271📖).

By grafting cranial paraxial mesenchyme and the 6 rostral somites, Couly et al (1992📖) were able to delineate precisely the respective contributions to the skull of the somites, paraxial mesenchyme and of the neural crest. As represented on 3.101👁️ for the chick, a region of the skull rostral to the extreme tip of the notochord can be distinguished; this region reaches the sella turcica and also a region caudal to this boundary. The former, the *achordal skull* is derived entirely from neural crest; the latter is derived from paraxial mesenchyme (cephalic or somitic)




in its ventromedial part and is termed the *chordal skull* (see also p. 287.





Apart from the skull, the cephalic neural crest is the origin of several other derivatives (see derivative table p. 145). The overlapping developmental capacities of paraxial mesenchyme and neural crest is illustrated by the *meninges*. These are derived from the neural crest at the prosencephalic level (di- and telencephalon) and from paraxial mesenchyme for the rest of the CNS (see p. 256.

The *connective tissue component* of the glands derived from the buccal and pharyngeal epithelium, e.g. *salivary*, *thyroid*, *parathyroid* and *thymus* (see p. 176), and the *tunica media* and *externa* of the large blood vessels arising from the heart, are of neural crest origin (see p. 314), as are the *carotid body type I* and *type II cells* and the *calcitonin producing cells* (C-cells) that develop in the ultimobranchial bodies.


Although mesectoderm and mesenchyme share several developmental potentialities, that of yielding *vascular endothelial cells* is the strict reserve of the *mesenchyme* (see angioblastic mesenchyme pp. 156 and 299). That is why grafts of quail cephalic neural crest in chick embryos produce bones and connective tissues of donor type, but the vascular endothelium of all the vessels irrigating these tissues derives from the angioblastic mesenchyme of the host.

Neural Crest and the PNS

Although the development of the spinal ganglia from neural crest has long been known, confusion existed regarding the origin of the PNS and the level of the neuraxis from which its various components emanate (Le Douarin & Teillet 1973; Le Douarin 1982). Using quail-chick chimeras, the fate maps of the PNS derivatives of the neural crest and of the placodal ectoderm, which contributes to the sensory ganglia of certain cranial nerves, were established (3.102.

A monoclonal antibody, NC1/HNK1, was found to label most avian neural crest cells at the time of their migration (Tucker et al 1984). It recognizes a glycosylated epitope carried by several surface molecules. Although it is not strictly specific for neural crest cells, even at migration time (the NC1/HNK1 epitope is lost by certain crest cells such as the mesectoderm, certain neurons and the melanocytes, but remains present on peripheral glia and neuronal subpopulations), the use of this antibody has been instrumental in showing that the *migration pattern* of neural crest cells in the trunk is **channelled by the somites**. It thus appears segmental although the crest cells exit uniformly along the whole neural tube. The crest cells are able to migrate, without impediment, between the somites and within the rostral sclerotomal half, but they cannot penetrate the caudal moiety of the sclerotomal mesenchyme (see p. 265). Thus the segmental distribution of the spinal and sympathetic ganglia is imposed on the neural crest cells by a prepattern that exists within the somitic paraxial mesenchyme (Rickmann et al 1985; Kalcheim & Teillet 1989.




Further experiments examined whether crest cells which were destined to form different parts of


the PNS (3.102 ) were already restricted in their developmental potential. This could occur prior to migration, or be imposed subsequently by environmental cues to which they were exposed during migration or at their final destination. Quail neural crest cells were either transplanted before the onset of migration into *heterotopic* sites in the chick host, or developing quail peripheral ganglia were 'back transplanted' into the neural crest migration pathway of the chick.

Vagal Neural Crest

This normally gives rise to enteric ganglia but was transplanted into the neuraxis at the level (somites 18–24) from which the adrenal medulla, sympathetic and sensory ganglia are derived. The grafted quail cells followed the migration pathway appropriate to the *recipient* site. The cells reached the target organs (sympathetic ganglia and adrenal medulla) of the host where they differentiated into catecholamine producing cells and not into the enteric cholinergic neurons that would have been their normal fate. Similarly, when a neural primordium of the adrenomedullary level was transplanted into the vagal area, the grafted neural crest cells migrated to the gut where they differentiated into enteric ganglia; thus they did not express the adrenergic phenotype.

The back-transplantation approach showed that all types of peripheral ganglia contain, besides their characteristic neuronal and glial cell types, precursor cells that do not differentiate in these sites but can be led to do so if they are provided with appropriate environmental cues. Thus adrenergic cells can arise from sensory ganglia and from enteric plexuses in which this phenotype does not normally exist.

Neural crest derived glial cells provide another example. A surface glycoprotein of the immunoglobulin-like superfamily, the Schwann cell myelin protein (SMP), was found to be expressed exclusively by myelinating and non-myelinating Schwann cells in vivo (Dulac et al 1992 ). Neither the enteric glia nor the satellite cells of the peripheral ganglia carry this marker. The control of SMP gene expression by environmental cues and particularly its inhibition by the microenvironment of the gut of the dorsal root ganglion was demonstrated experimentally by changing the environment of the various types of glial cells. Thus enteric glia and sensory ganglia satellite cells synthesize SMP when they are withdrawn from their normal environment and cultured in vitro (Dulac & Le Douarin 1991 ; Cameron-Curry et al 1993 

It appears, therefore, that *precursors* of virtually all the cell types that compose the PNS are **present at every level** of the neuraxis (3.102 ). Therefore, the apparent regionalization of the fate map reflects the diversity of the embryonic rudiments to which the neural crest cells migrate rather than intrinsic restrictions that exist in the neural crest cell population before migration.

Factors Promoting Neural Crest Survival

Both survival and differentiation of sensory neuronal progenitors have been shown to depend on factors provided by the neural tube at the time the neural crest cells aggregate to form the dorsal

root ganglia (E3–E4, i.e. days 3–4, in chick and quail embryos). Neurotrophins of the brain derived neurotrophic factor (BDNF) family along with the extracellular matrix protein, laminin, were shown to be responsible in this case (Kalcheim & Le Douarin 1986; Kalcheim et al 1987). Clearly other growth factors must play similar roles for the other types of neural crest derivatives. For example, basic fibroblastic growth factor (b-FGF) (Kalcheim 1989; Kalcheim & Neufeld 1990), insulin and insulin-like growth factor (IGF1) (Le Douarin & Smith 1988) were found to influence the differentiation of definite sets of neural crest derived cells at the early ontogenetic stages. Another example is provided by the *steel* factor and its receptor, c-kit, which play a decisive role in the differentiation of melanocytes (Williams et al 1992).

The Pluripotentiality of Neural Crest Cells Analysed in Clonal Cultures

Culture conditions can be provided which allow neural crest cells to grow and express their developmental potentialities, even when seeded as single cells (Sieber-Blum & Cohen 1980; Baroffio et al 1988, 1991). This can be achieved in a culture medium which, ideally and in contrast to in vivo conditions, exerts minimal or no selective pressures on these cells but allows them to express their differentiating capacities without restriction.

Experiments involving either cephalic or truncal neural crest cells at the migratory stage showed that most of them are pluripotent, in that they yield clones that contain multiple phenotypes revealed by various markers. Thus different types of neurons can be identified by their morphology and their content of neurofilament proteins, neuropeptides or of enzymes for the synthesis of neurotransmitters. Glial cells react with antibodies that recognize glial surface molecules in vivo, such as HNK1, and Schwann cell myelin protein; melanocytes contain their own marker, melanin, and mesectodermal derivatives can be recognized when they differentiate into cartilage nodules or into desmin containing cells (Baroffio et al 1988; Ito & Sieber-Blum 1991). The abilities of individual neural crest cells to proliferate and differentiate, however, are highly variable. Some give rise to two and others to three or more phenotypes and the size of the clones varies greatly. A few cells give rise to clones in which only one cell type (e.g. neuronal or glial) can be detected and these are believed to arise from monopotent, fully committed precursors. The cells which give rise to clones in which all representatives of the neural crest are present, including mesectodermal derivatives such as cartilage, are found only in the cephalic area. Mesectodermal derivatives are commonly found associated with neural, glial and melanocytic cell types. This shows that, at the time of crest cell emigration, mesenchymal and neurectodermal lineages are not segregated in the neural crest as they are in the ectoderm and the mesoblast after gastrulation.

Thus it seems that the neural crest contains *totipotent stem cells* that are analogous to the hemopoietic stem cells which give rise to the multiple blood cell lineages (Anderson 1989). Such putative stem cells are only rarely seen during the migratory phase of the cephalic neural crest. It will be interesting to see if their frequency is higher at earlier stages of crest cell ontogeny.

Stem cells are characterized by their capacity for self renewal. Such self renewal has been

demonstrated in rat neural crest (Stemple & Anderson 1992^[1]). In vivo labelling experiments in which chick premigratory single neural crest cells were either injected with a fluorescent dye (Bronner-Fraser & Fraser 1988^[2], 1989^[3]), or infected with retroviruses (Frank & Sanes 1991^[4]) revealed that both clone size and cellular composition were variable. These results led to the notion that crest cell populations that reach their migratory destination are highly pluripotent. Only a small part of these potentialities will be realized in each neural crest derivative. Cells whose fate does not fit with the conditions encountered in a precision location will either die or remain quiescent. The latter alternative was demonstrated in experiments where neural crest cells, withdrawn from their normal environment such as the gut (Rothman et al 1987^[5]), various types of sensory ganglia (Le Douarin & Smith 1988^[6]) or the skin (Richardson & Sieber-Blum 1993^[7]) and subjected to different conditions, gave rise to derivatives that do not exist in their tissue of origin. Thus these studies suggest plasticity of neural crest cell development and assign control of the patterning mainly to the embryonic territories that the crest cells colonize.

Ectodermal Placodes

The elevating neural folds formed during neurulation contain neural crest cells along most of the neural axis; these cells undergo epithelial/mesenchymal transition to acquire the migratory characteristics of crest cells. Other cells have been identified which originate in the neural folds but remain within the surface ectoderm after neurulation. These areas of neurepithelium within the surface ectoderm have been termed *ectodermal placodes*. Although the majority of the ectodermal placodes form nervous tissue, non-neurogenic placodes occur. After an appropriate inductive stimulus the local clusters of placodal cells remove themselves from the surrounding surface ectoderm either by *epithelial/mesenchymal transition* or by *invagination* of the whole placodal region to form a *vesicle* beneath the remaining surface ectoderm. *Neurogenic placodes* undergo both processes; paired *non-neurogenic placodes* invaginate to form the lens vesicles under the inductive influence of the optic vesicles (see p. 259^[8]).

The neural folds meet in the rostral midline adjacent to the buccopharyngeal membrane; this *rostral neural fold* does not generate neural crest but itself gives rise to the *hypophyseal placode*, i.e. the future Rathke's pouch (see p. 257^[9]), which remains within the surface ectoderm directly rostral to the buccopharyngeal membrane. The rostral neural fold also gives rise to the *olfactory placodes* (see p. 222^[10]), which remain as paired, laterally placed placodes, and to epithelium of the nasal cavity (3.100^[11]).

Further caudally, similar *neurogenic placodes* can be identified and divided into three categories, *ventrolateral* or *epibranchial*, *dorsolateral* and *intermediate* (3.103^[12]). The epibranchial placodes appear in the surface ectoderm immediately dorsal to the area of pharyngeal (branchial) cleft formation. The first epibranchial placode is located at the level of the first pharyngeal groove and contributes cells to the *distal (geniculate) ganglion* of the VIIth cranial nerve; the second and third epibranchial placodes contribute cells to the distal ganglia of cranial nerves IX (*petrosal*) and X (*nodose*) respectively. Generally these placodes thicken and cells begin to detach from the epithelium soon after the pharyngeal pouches have contacted the overlying ectoderm. Concurrently the neural crest cells reach and move beyond these lateral extensions of

the pharynx. Cells budding off placodes show signs of early differentiation into neurons including the formation of neurites (D'Amico-Martel & Noden 1983^[4]). Epibranchial placodes may have their origins in the neurons innervating the taste buds in fishes.

Dorsolateral placodes may be related evolutionarily to the sensory receptors of the lateral line system of lower vertebrates. They are represented by the *otic placodes*, located lateral to the myelencephalon; they invaginate to form otic vesicles which become the *membranous labyrinth of the ear*. Neurons of the VIIIth nerve ganglia arise by budding off the ventromedial aspect of the otic cup after which they can be distinguished in the *acoustic* and *vestibular ganglia*.

Intermediate between the epibranchial and dorsolateral placodes are the *profunda* and *trigeminal placodes*. In man the profunda and trigeminal placodes are fused to form a single entity. Prospective neuroblasts migrate from foci dispersed throughout the surface ectoderm lateral and ventrolateral to the caudal mesencephalon and metencephalon to contribute to the distal portions of the trigeminal ganglia. (For a lucid account of placodal development see D'Amico-Martel & Noden 1983^[4]).

Neuroglia

Glial cells which support neurons in the CNS and PNS derive from three lineages, the *neuroectoderm* of the neural tube, the *neural crest*, and *angioblastic mesenchyme*. In the CNS, cells of the proliferating ventricular zone give rise to *astrocyte* and *oligodendrocyte* cell lines, the first a supporting and reactive cell in the CNS, the second responsible for myelination in the CNS. After the proliferative phase the cells remaining at the ventricular surface differentiate into *ependymal cells* which are specialized in many regions of the ventricular system as circumventricular organs (Collins and Woollam 1981^[4]). In the peripheral nervous system, neural crest cells produce *Schwann cells* which myelinate peripheral nerves, and also *astrocyte-like support cells* in the enteric nervous system (see p. 235^[4]). Angioblastic mesenchyme gives rise to a variety of blood cell types including the circulating monocytes which infiltrate the brain as *microglial cells* later in development.

The ventricular zone lining the early central canal of the spinal cord and the cavities of the brain give rise to at least two types of cell: glial fibrillary acidic protein (GFAP) positive and negative; many of their proliferative progeny migrate into the intermediate zone. The negative cells form neuroblasts which differentiate into neurons, and the positive cells glioblasts which differentiate first to form *primitive radial* varieties, and then generations of astroblasts and oligodendroblasts; the latter mature into astrocytes and oligodendrocytes (for their mature morphology and hypothesized functional roles, see p. 940^[4]). The earliest glioblasts' radial processes extend both outwards to form the outer limiting membrane deep to the pia mater and inwards, forming the inner limiting membrane around the central cavity. Their cell geometry may provide contact guidance paths for subsequent cell migrations, both neuroblastic and glioblastic. As the glioblasts differentiate into primitive neuroglia some lose their connections with both inner and outer limiting membranes. They may partially clothe the somata (between presumptive synaptic

contacts) of neighbouring developing neuroblasts, or similarly enwrap intersynaptic surfaces of their neurites. (When many varieties of axon are involved, the encircling glial processes form internodal segments of myelin.) Further glial processes expand around intraneural capillaries as perivascular end-feet. Other glioblasts retain an attachment (or form new expansions) applied as pial end-feet to the innermost stratum of the meninges (pia mater)—this is the *pia intima* of some neurocytologists. Both strata may be termed *pia-glia*. In the developing rat brain, glioblasts which will develop into either oligodendrocytes or type-2 astrocytes are termed O-2A progenitors. They are known to express platelet derived growth factor (PDGF) receptors, unlike neurons, and can be stimulated to divide in culture by PDGF (Pringle et al 1992^[1]). Still other glioblasts remain lining the central canal and cavities of the brain as generalized or specialized ependymal cells, including tanocytes, but lose their peripheral attachments. In some situations, as in the anterior median fissure of the spinal cord, the ependymal cells retain their attachments to both the inner and outer limiting membranes. Thus, in addition to functioning as perineuronal satellites, the glia provide cellular channels interconnecting extracerebral and intraventricular cerebrospinal fluid, the cerebral vascular bed, the intercellular crevices of the neuropil and the cytoplasm of all neural cell varieties. (For further comments see pp. 937–940^[2]).

Microglia appear in the CNS after it has been penetrated by blood vessels and invade it in large numbers from certain restricted regions, whence they spread in what have picturesquely been called 'fountains of microglia', to extend deeply amongst the nervous elements.

Mechanisms of Neural Development

Segmentation in the Nervous System

Segmentation is conspicuous in man and other vertebrates in the serial arrangement of the vertebrae and axial muscles and in the periodicity of the spinal nerves. In the last century, the possibility that the neural tube might be divided into *segments* or *neuromeres* was entertained but some contended that bulges observed in the lateral walls of the neural tube were artifacts, or caused by mechanical deformation of the tube by adjacent structures. Recent years have seen a resurgence of interest in this subject, leading to a detailed evaluation of the significance of neuromeres. A series of eight prominent bulges which appear bilaterally in the rhombencephalic wall early in development have been termed *rhombomeres* (3.104^[1]). (While the term neuromere applies generally to putative 'segments' of the neural tube, the term rhombomere applies specifically to the rhombencephalon.) Rhombomeres have now been shown to constitute crucial *units of pattern formation*, based on the fact that many aspects of the patterning of neuronal populations and the elaboration of their axon tracts conform to a segmental plan. Domains of gene expression, for example those of the *Hox b* genes and the transcription factor *Krox 20*, about rhombomere boundaries, and perhaps most importantly, single cell labelling experiments have revealed that cells within rhombomeres form segregated non-mixing populations. The neural crest also shows intrinsic segmentation in the hindbrain, being segregated into streams at its point of origin in the dorsal neural tube (Lumsden et al 1991^[2]). This may represent a mechanism whereby morphogenetic specification of the pre-migratory neural crest cells is conveyed to the pharyngeal arches. Although these segmental units lose their

morphological prominence with subsequent development, they represent the fundamental ground plan of this part of the neuraxis, creating a series of semi-autonomous units within which local variations to patterning can then develop. The consequences of early segmentation for events later in development, such as the formation of definitive neuronal nuclei within the brainstem and of peripheral axonal projections remains to be explored.

The appeal of segmentation as a fundamental principle in neural development rests on its theoretical attractiveness as a way of simplifying developmental problems, superimposing variations in regional identity on a basal, segmented pattern. In higher vertebrates, the diencephalic anlagen of various regions of the thalamus are found to exhibit distinctive patterns of histochemical staining, and to constitute domains of cell lineage restriction (Figdor & Stern 1993). The organization of the telencephalon is also reflected in domains of gene expression that abut sharp boundaries (reviewed in Puelles & Rubinstein 1993; Boncinelli 1994). However, substantial evidence that these regions employ segmentation in the same manner as the hindbrain has yet to accrue. It is also possible that while in the hindbrain rhombomeres are early units of patterning which are later obscured, those in the forebrain are later structures that constitute the definitive functional units. Recently, there have been many reports that the domains of expression of many presumed regulatory genes have sharp boundaries within the forebrain, some of which correspond with sulci identified in anatomical studies. Thus the subject of segmentation and its possible role in neural patterning in many brain regions is bound to continue to attract attention (see below).

The importance of *intrinsic segmentation* in the hindbrain is underlined by the absence of overt segmentation of the adjacent paraxial mesenchyme. While the somites are prominent segmented entities within the trunk, the mesenchyme of the head appears to be a single continuous block. Somitomeres have been identified lateral to the notochord by scanning electron microscope (Meier 1981; Meier & Packard 1984; see p. 154), but not all are convinced of their veracity. In the trunk, the sclerotomal mesenchyme (see p. 155) plays a role in segmenting the motor outflow and the emigration of the neural crest, and displays molecular heterogeneity. There is no evidence for this in the head; instead segmental properties are manifest in the neurectoderm. Moreover, ectomesenchyme cells seem to be specified with respect to their position prior to migration (Noden 1983a, b), and to be capable of patterning other structures, e.g. the muscles, in the arches into which they migrate. The emigration of neural crest cells from a particular axial level to the outlying arch is followed by axonal outgrowth of motor neurons from the same axial level, thus furnishing a mechanism whereby segmentation in the tube may be matched to that of the outlying structures.

In the spinal cord, however, there is no firm evidence for intrinsic segmentation. Instead, segmentation of the neural crest, motor axons, and thus eventually the spinal nerves, is dependent on the segmentation of the neighbouring somites. Experimental removal or intercalation of somites results in corresponding disturbances of pattern in these nervous structures (Keynes & Stern 1984, 1988). Both neural crest cell migration and motor axon outgrowth occur through only the rostral and not the caudal sclerotome of each somite. The segmental emergence of crest cells that results ensures that dorsal root ganglia form only at intervals. The caudal sclerotome possesses inhibitory properties that deter neural crest cells and

motor axons from entering (Davies et al 1990). This illustrates the general principle that the nervous system is closely interlocked, in terms of morphogenesis, with the 'periphery', i.e. surrounding non-nervous structures, and each is dependent upon the other for its effective structural and functional maturation.






Patterning of the Brain and Spinal Cord






For more than a century experimental strategies have been devised to elucidate mechanisms that operate during the development of the nervous system. Whilst much has been established, answers to many fundamental questions still remain obscure. In recent years, the best efforts to understand the development of 'higher' vertebrates has perhaps come from work on the amphibian and chicken embryos, in which organisms embryological, biochemical and molecular techniques can be combined to greatest effect (see Detwiler 1936; Spemann 1938; Weiss 1950; Hughes 1968; Gottlieb 1974).

The generation of neural tissue involves an inductive signal from the underlying chordamesoderm (p. 295). Neural induction was discovered during experiments on the amphibian embryo by Spemann in 1925. In the absence of this signal, ectoderm cells form epidermis; in its presence they form nervous tissue. Despite nearly 70 years having elapsed since the discovery of neural induction, the identity of the signal remains a mystery. Under experimental conditions a disappointingly wide variety of non-specific substances have been found to have a neural inductive influence in salamander and newt embryos, implying that the ectoderm is finely balanced between a neural and an epidermal fate (Spemann 1938; Hamburger 1988). Molecular biological approaches have, however, added impetus to these studies, and have identified some potential positive and negative regulators of neural induction. This work has concentrated on the frog embryo, *Xenopus laevis*, whose ectoderm is not so easily neuralized. The molecule *activin*, belonging to the transforming growth factor beta (TGF β) family, is a known inducer of *mesoblastic tissue* (Smith 1987) and has been implicated in suppressing neural induction since in frog embryos, containing a truncated and non-functional activin receptor, neural tissue is overproduced (Hemmati-Brivanlou & Melton 1992). (For a discussion concerning the use of the term, mesoblast, see p. 93.) This finding might imply that neural differentiation is a default pathway, which activin normally acts to suppress. The suppressive effect of activin can only be blocked by *follistatin*, though this molecule is itself only a weak neural inducer (Hemmati-Brivanlou et al 1994). In addition another factor, *noggin*, has recently been isolated that can induce neural tissue from isolated ectoderm (Smith et al 1993). Hepatocyte growth factor/Scatter factor may also have the ability to induce neural tissue (Stern et al 1990). It is likely that in the future other candidate neural inducers will continue to be isolated; to elucidate the role of these various factors will require their inductive effect in culture to be placed in the context of their distribution and action in normal embryogenesis.

The first elements of the emerging neural pattern may be a **consequence** of neural induction. Neural induction is thus not solely a process that ensures neural differentiation, it is also involved in determining the regional pattern of the nervous system, before and during neural tube closure. Since neural inducing substance(s) have largely remained a mystery, attention has focused instead on the response of the induced neural tissue. Now, the abundance of

region-specific molecular markers has renewed attempts to unravel the signals in regional induction.

Genes such as the *Hox* and *Pax* gene families, which encode transcription factor proteins and their homologues, cloned invertebrates, show intriguing expression patterns within the nervous system. Genes of the *Hox-b* cluster, for example, are expressed throughout the caudal neural tube, and up to discrete limits in the hindbrain that coincide with rhombomere boundaries. The ordering of these genes within a cluster on the chromosome (5'–3') is the same as the caudal to rostral limits of expression of consecutive genes. This characteristic pattern is surprisingly similar in fish, frogs, birds and mammals. Persuasive evidence is now accruing that *Hox* genes play a role in patterning not only of the neural tube but of much of the head region, consistent with their expression in neural crest cells, and within the pharyngeal arches (see p. 287 ). While these genes demarcate regions up to and including the hindbrain, those of the *Dlx*, *Otx* and *Emx* families are expressed in different rostrocaudal domains within the forebrain. Some *Pax* genes, on the other hand, are expressed in different dorso-ventral domains within the neural tube. *Pax-3* is expressed in the alar lamina, including the neural crest, while *Pax-6* is expressed in the intermediate plate. Both *Hox* and *Pax* genes have restricted expression patterns with respect to the rostrocaudal and the dorsoventral axes of the neural tube, consistent with roles in positional specification. (For reviews of the expression patterns of these genes see McGinnis & Krumlauf 1992 ; Krumlauf et al 1993 ; Puellas & Rubinstein 1993 ; Chalepakakis et al 1993 .

The expression of *Hox* genes and other molecular markers have been used to investigate the mechanisms of regional induction, particularly in the frog embryo. This work indicates that specification of the rostrocaudal axis probably occurs somewhat earlier than that of the dorsoventral axis. Earlier in the century, embryologists concentrated on the idea that regionalization of the mesoblast imposed a similar mosaic of positional values on the overlying neural plate. In amphibia, transplantation of various regions of mesoblast beneath the neural plate showed a regional induction. Caudal mesoblast induced spinal cord, whereas rostral mesoblast induced brain, as assessed by the morphology of the neuroepithelial vesicles (Mangold 1933 ). Supporting evidence for this hypothesis using contemporary methods has been scarce, however. One example is the finding that rostral mesoblast induces expression of the *engrailed* marker (expressed in the rostral neural tube) more frequently than does caudal mesoblast. Presently, the consensus view regarding the rostral axis is that the neural plate has considerable self-organizational properties. Signals from the mesoblast are undoubtedly required to elicit neural induction, but it is not necessary for regions of the mesoblast to underlie their corresponding neural counterpart in register to evince regionalization. Thus, for example pieces of tissue isolated prior to gastrulation in which the mesoblast and ectoderm lie in linear array exhibit a surprisingly accurate pattern of expression of *Hox* genes and other positional markers (Doniach et al 1992 ). Supporting evidence comes from experiments on amphibian exogastrulae, in which gastrulation fails and the mesoblast never underlies the neural plate. Exogastrulae exhibit the expression of a range of neural markers (Dixon & Kintner 1989 ; Ruiz i Altaba 1992 ). This implies that a signal travels in the *plane* of the neur ectoderm, a possibility originally noted by Spemann (1938 ). In the same experimental system, patterns of expression of a *Dlx* gene in the forebrain were less faithfully rendered. The addition of prechordal mesoblast to the tissue pieces produced a better representation of the pattern, suggesting that patterning in more

cranial regions requires additional signals to that travelling by the planar route (Papalopulu & Kintner 1993). Consistent with this is the observation that eyes (also forebrain structures) were never found in planar recombinations of ectoderm and mesoblast or in exogastrulae (Ruiz i Altaba 1992). Vertical apposition of the mesoblast is also required to promote differentiation of the floor plate and motor neurons in the dorsal part of the neural tube.

Whilst craniocaudal positional values are probably conferred on the neuroepithelium at the neural plate or early neural tube stage, dorsoventral positional values may become fixed later. The development of the dorsoventral axis is heavily influenced by the presence of the underlying notochord. The notochord induces the ventral midline of the neural tube, the floor plate (van Straaten et al 1988; Placzek et al 1990, see above). This specialized region consists of a strip of non-neural cells with distinctive adhesive and functional properties. Notably, it produces a chemoattractant that specifically directs the growth of commissural axons ventrally, to cross the floor plate and project contralaterally (Tessier-Lavigne et al 1988). Notochord and floor plate together participate in inducing the differentiation of the motor columns (Yamada et al 1991). Motor neuron differentiation occurs early, giving some grounds for the idea of a ventral to dorsal wave of differentiation. The notochord/floor plate complex may also be responsible for allotting the values of more dorsal cell types within the tube (3.105). For example, the dorsal domain of expression of *Pax-3* extends more ventrally in embryos experimentally deprived of notochord and floor plate, while grafting an extra notochord adjacent to the dorsal neural tube leads to a repression of *Pax-3* expression (Goulding et al 1993). Investigation of the conditions required for the generation of other neuronal groups has been hampered by the lack of cell-type-specific markers.

Evidence for the involvement in neural development of *Hox* and *Pax* genes has come from studies of animals mutant for members of these gene families. In the pharyngeal region of the head individual *Hox* genes are expressed within matching domains of the brain, the neural crest and the adjacent pharyngeal arches (see p. 238), giving rise to the idea that this might embody a code for patterning the region—*Hox code*. Transgenic 'knockout' mice have been generated by targeted disruption of *Hox* genes using homologous recombination in embryonic stem cells. Mice rendered deficient for *Hox a-3* showed defects in neural crest derived ganglia and derivatives of the third and fourth pharyngeal arches, corresponding with the pattern of normal gene expression (reviewed in Hunt & Krumlauf 1991). Knockouts of *Hox a-1* produced mice that showed disruptions in patterning of the hindbrain rhombomeres and also in the cranial ganglia, in a manner that reflected disruption of a transient and early phase of expression of the gene (reviewed in Wright 1993). Disruption of *Pax* genes also leads to developmental abnormalities in mice, and in the case of at least three genes these may be related to human diseases. For example, the gene *Pax-3* was found to have the same chromosomal localization as the mouse mutation *Sploch* and the affected locus in the human *Waardenburg's syndrome*, both of which are characterized by neural crest disturbances with pigmentation disturbances and occasional neural tube defects. Mice heterozygous for a mutation in *Pax-6* have underdeveloped eyes; mutations in the human *Pax-6* gene lead to aniridia, a condition characterized by complete or partial absence of the iris and also affecting the cornea, lens, retina and optic nerve (reviewed in Chalepakidis et al 1993; see also p. 337).

Patterning of the Branchiorhombomeric Region

Intrinsic genetic control of the patterning of their derivatives appears also to exist in the neural crest cells in the branchiorhombomeric (the rhombencephalon plus the lateral pharyngeal arches) region of the body. The view that the neural crest itself possesses its own patterning capacities is supported by discoveries regarding the metameric organization of the hindbrain in vertebrates and its relation with the genetic control of its development by genes containing homeobox sequences with high degrees of homology with that of the *Drosophila Antennapedia* (*Antp*) homeogene (Hunt & Krumlauf 1992📖).

The expression of genes in the *Hox a, b, c and d* clusters (previously *Hox-1* to 4) has been analysed in detail in the hindbrain and corresponding neural crest in the mouse (3.106👁️). With few exceptions, the *Hox* genes expressed in the rhombomeres are **also** expressed in the neural crest at the same transverse levels. When neural crest cell migration is completed the same set of *Hox* genes is activated in the surface ectoderm. Using the quail–chick marker system, Couly and Le Douarin (1990📖) distinguished, in the branchiorhombomeric region, a metameric disposition of ectodermal stripes or *ectomeres*. These include the rhombomeres, the neural folds which produce neural crest cells, and the surface ectoderm covering the pharyngeal arch colonized by the neural crest cells, arising from the same level. Taken together, these observations suggest that the positional information that controls pharyngeal arch patterning is first contained in the rhombencephalon and then transmitted to the pharyngeal area via neural crest cells. The fact that positional information is controlled by a combination of *Hox* gene expression was further demonstrated by targeted mutations in the mouse.

Disruption of *Hox a-3* gene mimics the Di George's syndrome, a congenital human disorder characterized by the absence (or near absence) of the thymus, parathyroids and thyroid, by the hypotrophy of the wall of the arteries derived from the aortic arches, and by subsequent conotruncal cardiac malformations. The *Hox a-3* nul mice also show reductions in the skeletal development of the jaws and hyoid cartilage and in the muscular and connective tissues of the pharynx and the tongue. The rhombencephalic neural crest provides the entire set of mesenchymal cells to the thymic, thyroid and parathyroid rudiments, and constitutes the whole musculoconnective wall of the arteries arising from the aortic arches (see p. 314📖). Moreover, it has been established (Auerbach 1960📖, 1961📖) that thymic histogenesis depends strictly upon induction of the epithelium by the mesenchyme. Similar interactions are critical as well for the other glandular structures of the buccopharyngeal area (Le Douarin 1968📖). It is therefore understandable that a developmental failure of the hindbrain neural crest accounts for all the Di George's syndrome anomalies and for most of those of the *Hox a-3/Hox a-3* mice.

In experimental mutation of the *Hox a-1* gene (Thomas & Capecchi 1990📖; McMahon & Bradley 1990📖; Lufkin et al 1991📖), the defects observed concerned the neural (and not the mesectodermal as for *Hox a-3*) derivatives of the neural crest and of the CNS at the level of rhombomeres 4–7. The morphogenesis of different neural crest derivatives corresponding either to the mesectodermal and/or the neural lineages therefore appear to be influenced by **different** sets of regulatory genes. Disruption of the *Hox a-2* gene has recently been shown to result in the

homeotic transformation of skeletal elements derived from the second pharyngeal arch into more anterior structures corresponding to the 1st arch. This supports the contention that a combination of *Hox* genes (*Hox code*) is responsible for the patterning of neural crest hypobranchial derivatives and providing them with regional specification along the cranio-caudal axis of the body.


Histogenesis of the Neural Tube

The classic view of neural tube histogenesis was first propounded by Wilhelm His in 1890^[1] and soon gained quite wide acceptance (e.g. Ramón y Cajal 1911^[2]). He proposed that subclasses of germinal cells were progenitors of particular classes of neurons and glial cells. Thus, almost from the first the wall of the tube was *stratified* and contained a variety of distinct cell types—*spongioblasts*, *neuroblasts* and *germinal cells*. Contemporaneously, the opposing view, that of *multipotential progenitors*, was also put forward (Vignal 1889^[3]; Koelliker 1887^[4]).

The round deeply placed germinal cells or *medulloblasts* (Glees 1963^[5]) were regarded by His as undifferentiated stem cells, which by repeated division gave further generations of both spongioblasts (glial progenitors) and neuroblasts. The primitive spongioblasts, originally elongate cells attached to both limiting membranes with their nuclei in the ventricular zone, were considered to differentiate into a number of *sustentacular cell* varieties. By losing contact with one or both limiting membranes, they would then differentiate into either astroblasts and astrocytes or oligodendroblasts and oligodendrocytes, or, retaining an internal attachment, into the definitive ependymal cells which line the central canal, and regionally specialized tanocytes (p. 939^[6]). The neural precursors, one of the cell types found in the mantle zone (intermediate zone), differentiated into the wide array of neurons. On this view, therefore, which constituted a *polyphyletic theory of neurogenesis*, the early neural epithelium was regarded as a *heterogeneous* grouping of cells with their various derivatives, developing **simultaneously**.

An opposing view, expounded by Schaper (1897^[7]), interpreted the mitotic figures as synonymous with the neuroepithelial cells, simply seen in a different location and in different phases of the cell cycle. Not until the meticulous cytological studies of Sauer (1935a^[8], b^[9], 1936^[10]) did this theory gain wide acceptance. He maintained that the early neural epithelium, including the deeply placed ventricular mitotic zone, consists of a *homogeneous* population of pluripotent cells, the varying appearances merely reflecting different phases in a *proliferative cycle*, the sequence being termed by Sauer *interkinetic migration*. This proposal was essentially a *monophyletic theory of neurogenesis*. In subsequent years much experimental evidence based upon colchicine studies (e.g. Watterson et al 1956^[11]), spectrophotometric nuclear analysis (e.g. Sauer & Chittenden 1959^[12]) and upon autoradiographic studies following the distribution of cells at varying times after labelling with tritiated thymidine (e.g. Sidman et al 1959^[13], Sidman 1970^[14]; Fujita 1963^[15]; Fujita & Fujita 1963^[16]) and ultrastructural studies (e.g. Hinds 1971^[17]) have supported the latter proposition. The scheme amplified by Fujita in a series of later publications is illustrated in 3.107^[18]. The ependymal layer of earlier workers (termed by Fujita the *matrix layer*) is considered to be populated by a single basic type of *progenitor cell* and to exhibit three 'zones' (the M or mitotic, the I or intermediate, and the S or synthetic zones). As they pass through a complete intermitotic and mitotic cycle, the matrix cells show an 'elevator movement'

progressively approaching and then receding from the internal limiting membrane. DNA replication occurs whilst the cells are extended and their nuclei occupy the S zone; they then enter a premitotic resting period whilst the cells shorten and their nuclei pass through the I zone. The cells now become rounded close to the internal limiting membrane (in the M zone) and undergo mitosis; thereafter they elongate again, their nuclei passing through the I zone during the postmitotic resting period, finally to enter the synthetic zone once again. The cells so formed may then either start another *proliferative cycle* or migrate outwards (i.e. radially) and differentiate into neurons as they approach and enter the adjacent stratum; possibly this differentiation is initiated as they pass through the I zone during the postmitotic resting period. The proliferative cycle continues with the production of clones of neurons, but this process eventually declines and is superseded by the production of *ependymal cell* and *macroglial cell* varieties.

Cumulative tritiated thymidine labelling studies have shown that an initial period of *symmetric division* is followed by a period of *asymmetric division*. At the last division, of course, two postmitotic daughters are produced. Later, the progeny of some of these divisions move away from the ventricle to form an intermediate (mantle) zone of neurons; others form a *subventricular zone* between the ventricular and intermediate layers, there continuing to multiply to provide further generations of neurons and glia. Both of these cell types subsequently migrate into the intermediate and marginal zones but in some regions of the nervous system (e.g. the cerebellar cortex, p. 261 ) some mitotic subventricular stem cells migrate across the entire neural wall to form a *subpial population*, thus establishing a new zone of cell division and differentiation. Many cells formed in this site remain subpial in position but others migrate back towards the ventricle through the developing nervous tissue, finishing their migrations in various definitive sites where they finally differentiate into neurons and macroglial cells.









It should be emphasized here that differences of opinion persist (and have increased in the last decade), concerning the precise details of these processes and the possible mechanisms involved. Further, considerable confusion has obtained between different neuroembryologists in relation to the **terminology** to be adopted for the various '**layers**' or '**zones**' at different times and places in the developing neural tube. An international group of neurocytologists (the Boulder Committee 1970 ) proposed a less ambiguous nomenclature which is increasingly but not universally adopted. They termed the early pseudostratified neuroepithelium, in which the 'elevator movement' occurs, the *ventricular zone* (its further development is summarized below, on page 219 , and in 3.95 ). For an excellent review of the biological and terminological problems posed, and an extensive bibliography to the data indicated, consult Berry (1974 ) and for further comments on the natural history of neurons see page 921 .

Figure 3.95  summarizes the main stages of development of the neural tube and the nomenclature as **initially** proposed by the Boulder Committee (1970 ) the complete series of stages A–E applying to the cerebral neocortex: only stages A–C are relevant in the context of the spinal cord. As detailed in the caption, it will be seen that the early pseudostratified *ventricular zone* (V) is followed by the sequential appearance of *marginal* (M), *intermediate* (I), *subventricular* (S) zones and, concurrently with the latter, the early *cortical plate* (CP) (see p. 252 ). It is unfortunate that this use of term **zone** for these major ontogenetic strata does **not**










correspond to the M, I and S zones of Fujita detailed above (and in 3.107 ) , the latter applying to subdivisions of the early neural tube only. More than a decade of research employing technical innovations induced Rakic (1982 ) to suggest a series of additional features and modifications of the original Boulder proposals. These are mentioned at various points in the text, and summarized in relation to the neocortex on page 252  and in 3.123A-G .

Figure 3.70  (based on the work of Berry & Rogers 1965 ; Berry 1974 ) may be noted here for comparison with 3.123 ; it stems from labelling studies on the *pattern of migration* of neuroblasts in the developing neocortex in the rat and will be briefly mentioned later on page 253  et seq.




















The following decade, and to the time of writing, has seen accelerating, enthusiastic application of newer, more refined techniques to these and related problems: in particular immunohistochemistry and computer analysis of neurocellular geometry. This has resulted in reappraisal of some foregoing conclusions. The cytokinetics of the early pseudostratified neuroepithelium ('elevator' movement, or interkinetic migration) has been substantiated, as has the establishment of the principal zones proposed by the Boulder Committee. However, the latter has been modified (at least in the forebrain) by the recognition of an additional (though transient) stratum deep to the early cortical plate—the *subplate zone* (SP), see page 253 —and many further features of cortical and subcortical maturation. Throughout most of the neuraxis, proliferative cell cycles are limited to the ventricular (V) and subventricular (S) zones, but their homogenous nature has been challenged. Immunocytochemical methods for demonstrating glial fibrillary acidic protein strongly suggest that **at least two** cell varieties are present in the early tubal neuroepithelium, perhaps in a precise mosaic pattern. (This may be classified as an *oligophyletic theory of neurogenesis*.) The early disposition of radial glial fibres is proposed by some to have a profound morphogenetic significance. The latter concerns not only their spatially ordered array, but the probability that they provide migration paths, by contact guidance, for at least the earlier generations of migratory neuroblasts, thus specifying their destinations. Neuronal geometry is further considered on pages 237 , 243 , 252 . (See bibliographic reviews in Berry 1974 , 1982 ; Schmitt & Worden 1979 ; Berry et al 1980a , b , c , d ; Levitt & Rakic 1980 ; Rakic & Goldman-Rakic 1982 ; Smart 1982 , 1983 .)

Figure 3.108 , based on and slightly modified from Rakic (1981 , 1982 ) , is a simplified analysis of earlier and current theories of some cell varieties and lineages in neural tube histogenesis. In a recent presentation (Levitt et al 1981 ) some aspects of all earlier theories have been incorporated, others rejected. See caption and text above for further comment.

Lineage in the Nervous System

Neurons come from two major embryonic sources: *central neurons* originate from the neural plate and tube whereas *ganglionic neurons* originate from the neural crest and placodes. The neural plate also provides ependymal and macroglial cells, while from the neural crest arise inter alia peripheral Schwann cells and chromaffin cells. Recently many difficulties in determining the origins and lineages of cells in the nervous system have been resolved by the use of

autoradiography, microinjection or retroviral labelling of progenitor cells and cell culture.

During development, neurons are formed first, followed by glial cells. The timing of events differs in various parts of the CNS and between species. Most neurons are formed prenatally in mammals but some postnatal neurogenesis does occur, as in the case of the small granular cells of the cerebellum, olfactory bulb and hippocampus, and neurons of the cerebral cortex. Gliogenesis continues after birth in periventricular and other sites. Autoradiographic studies show that different classes of neurons develop at specific times. Large neurons such as *principal projection neurons* tend to differentiate before small ones such as *local circuit neurons*. However, their subsequent migration appears independent of the times of their initial formation. Neurons can migrate extensively through populations of maturing, relatively static cells, to reach their destination; for example, cerebellar granule cells pass through a layer of Purkinje cells en route from the external pial layer to their final, central position. Later, the final form of their projections, cell volume and indeed their continuing survival depend on the establishment of patterns of functional connection (p. 255). For an account of glial cell development see page 225.

There are many questions regarding the factors determining the lineage of neural cells. For example, do individual progenitor cells give rise to both neurons and glia, and/or to different neuronal subtypes? Are there defined fields of progenitor cells that give rise to different regions of the CNS? Resolving these questions involves labelling a single progenitor cell, allowing development to proceed and then recording the positions and phenotypes of all labelled progeny, using specific antibodies or precise morphological criteria (e.g. at electron microscope level). Whilst in amphibia and avia direct cell injection of fluorescent lineage tracers is feasible, this is so far impossible in mammals, owing to the inaccessibility of the embryos in utero. Instead, lineage analyses have relied on the infection of progenitor cells in the ventricular zone of rodent embryos using replication defective retroviruses (Sanes et al 1986; Price et al 1987). The retroviral DNA incorporates into the genome of the infected cell providing an indelible marker that is transmitted to all progeny during cell division, but not to surrounding cells. Initial infective events are random, affording no control over the number or location of cells infected. Thus, defining single clones at later developmental times, when cell mixing may have taken place, is problematic. However, both the direct injection and retroviral techniques have yielded some information about lineage in various regions of the nervous system. In the optic tectum of avia, a single cell can give rise to **both** neurons and glia with diverse forms (Galileo et al 1990). A similar pattern was observed for the avian spinal cord, in which clones contained motor neurons and a range of other neurons such as interneurons and preganglionic autonomic, along with glia and ependymal cells (Leber et al 1990). Similarly, for the retina, experiments on rodents show that labelled cells produce a range of derivatives, consisting of any combination of rods, bipolar cells, amacrine cells and Muller glia (Turner & Cepko 1987). Furthermore, in the retina, cells were able to yield more than one cell type up to their terminal division. It should be noted that Muller glia resemble astrocytes, but are significantly different by morphological and molecular criteria; typical astrocytes are present in the retina but they have a different lineage, migrating from the optic nerve.

By contrast, information on lineage within the cortex has suggested some degree of lineage

restriction. In one study, the majority of clones consisted of either neurons or glia (Walsh & Cepko 1992📖), while other authors found clones composed of either astrocytes, neurons or oligodendrocytes, or a mixture of the last two (Price & Thurlow 1988📖). An electron microscope study of the synaptic morphology and ultrastructure of retrovirally-marked cells has reported separate clones of pyramidal and non-pyramidal neurons, as well as of astrocytes and oligodendrocytes (Parnavelas et al 1992📖). These studies therefore tend to the conclusion that there are **separate** progenitors for different cell populations. There are two reasons why this conclusion might be called into question, however. First, this impression might be gained if the information gathered reflected events late in the lineage tree. Multipotent precursor cells might exist at early stages, but might not be detected, since the technical difficulties of introducing virus into the fetal ventricles precludes these experiments before E12 in the mouse or E14 in the rat. Secondly, recent studies suggest a much higher degree of cell dispersion tangentially within the cortex than has been suspected, making definitions of clonality problematic (see p. 255📖). Interestingly, a study in which progenitor cells were labelled in the chick hindbrain by direct cell injection yielded large clones of cells mostly with identical phenotypes and axonal projection patterns, implying early assignment of cell fate in this region.

Axonal Growth and Guidance

Initially neuroblasts are rotund or fusiform, their cytoplasm containing a prominent Golgi apparatus, many lysosomes, glycogen and numerous unattached ribosomes (Tennyson 1969📖). As maturation proceeds cells send out fine cytoplasmic processes; these contain neurofilaments, microtubules and other structures, often including centrioles at their bases where microtubules form (p. 31📖). Internally, endoplasmic reticulum cisternae appear and attached ribosomes and mitochondria proliferate but the glycogen content progressively diminishes. One process becomes the axon and other processes establish a dendritic tree. Axonal growth, studied in tissue culture, may be as much as 1 mm per day.

Ramón y Cajal (1890📖) was the first to recognize that the expanded end of an axon—the *growth cone*—is the principal sensory organ of the neuron. Subsequently, the growing tips of the neuroblasts have been studied in tissue culture (Harrison 1910📖; Speidel 1932📖, 1933📖, Pomerat et al 1967📖; Bray 1982📖; Letourneau 1985📖). Classically, the growth cone has been described as an expanded region that is constantly active, changing shape, extending and withdrawing small filopodia and lamellipodia that apparently 'explore' the local environment for a suitable surface along which extension may occur (Tennyson 1970📖; Bunge 1976📖; Pfenninger & Rees 1976📖; see 'Cell motility', p. 43📖). These processes are stabilized in one direction, determining the direction of future growth, and following consolidation of the growth cone, the exploratory behaviour recommences. This continuous cycle resembles the behaviour at the leading edge of migratory cells such as fibroblasts and neutrophils. The molecular basis of this behaviour is the scaffolding of microtubules and neurofilaments within the axon. Growing neuroblasts have a cortex rich in actin, associated with the plasma membrane, and a core of centrally-located microtubules and sometimes neurofilaments. The assembly of these components, along with the synthesis of new membrane, occurs in segments distal to the cell body and behind the growth cone, though some assembly of microtubules may take place near the cell body.

Indispensable to growth cone extension are products and organelles synthesized within the cell bodies that are passed outwards by proximodistal *axoplasmic flow* along the axons. Bulk axoplasmic flow was first postulated following the experimental construction of nerves (Weiss & Hiscoe 1948), and since that time many intricate analyses of fast and slow components of *bidirectional flow systems* within axons have been made (Lubinska 1964, and see (Neurosciences Research Programme 1968). The driving force of growth cone extension is uncertain. One possible mechanism is that tension applied to objects by the leading edge of the growth cone is mediated by actin, and that local accumulations of F-actin redirect the extension of microtubules. Under some culture conditions, growth cones can develop mechanical tension, pulling against other axons or the substratum to which they are attached. Possibly, tension in the growth cone acts as a messenger to mediate the assembly of cytoskeletal components. Adhesion to the substratum appears to be important for consolidation of the growth cone and elaboration of the cytoskeleton in that direction. However, growth is not simply proportional to adhesion, and axonal growth and guidance are likely to depend on a fine balance of cell surface and extracellular matrix molecules. For reviews on morphology, motility and directional growth of axons and their growth cones, consult James (1974), Rakic (1971a, b, 1981, 1982), Bentley and O'Connor (1994).

During development, the growing axons of neuroblasts navigate with precision over considerable distances, often pursuing complex courses to reach their targets. Eventually they make functional contact with their appropriate end organs (neuromuscular endings, secretomotor terminals, sensory corpuscles or synapses with other neurons). During the outgrowth of axonal processes from ventral motor neuroblasts to reach presumptive myoblasts in the limb buds, the earliest nerve fibres are known to cross appreciable distances over an apparently virgin landscape occupied by loose mesenchyme. A central problem for neurobiologists, therefore, has been understanding the mechanisms of axon guidance.

A vast research literature is now devoted to the issues of axonal pathfinding, e.g. '*The nerve growth cone*' edited by Letourneau et al (1990). Only the main ideas can be touched on here. Over the years two principal theories have emerged concerning the directional growth of nerve fibres—the *neurotropism* or *chemotropism hypothesis* of Ramón y Cajal (1919) and the principle of *contact-guidance* of Weiss (1941). The former, based initially upon observations on the innervation of epithelia, proposed that growing fibres were guided by some form of attraction, presumably chemical, which emanated from the target area to be innervated. The second view denied the existence of such attractive forces and, based upon many series of tissue culture experiments, held that pioneer axons were guided to their destination by preferential growth along pathways dictated exclusively by the structures with which the growth cone was in direct contact. After spending most of the century in the wilderness, however, the chemotropism idea of Ramón y Cajal has recently been borne out with relation to several aspects of neural development. The salient feature of chemotropism is that growth cones act as **sensors** to concentration gradients of molecules in the environment, and grow up the gradient towards the source, i.e. the target. This situation can be mimicked in vitro using an explant culture system in which tissue explants (neuroblasts of interest and target tissue) are placed at a distance in a three-dimensional collagen gel. In this system, stable gradients of substances diffusing from the target tissue may be established. Using this system, the developing epithelium of the face in the

mouse, for example, has been shown to originate a chemoattractant that lures sensory afferents of the trigeminal system (Lumsden & Davies 1983, 1986) (3.109). In addition, the floor plate of the developing spinal cord has been found to exert a chemotropic effect on commissural axons that later cross it (Tessier-Lavigne et al 1988), while the developing pons produces a chemoattractant that elicits collateral budding from descending corticofugal fibres (Heffner et al 1990). Another possibility is that diffusible factors could also mediate chemorepulsion, deflecting growing axons from inappropriate areas, as has been shown to occur for olfactory axons, which avoid explants of septal tissue in culture (Pini 1993).

There is no doubt, however, that contact guidance mechanisms operate in parallel with neurotropism. Physical cues in the pathway may play a role, such as the pattern of spaces in the spinal cord of the newt, hypothesized to constitute a 'blueprint' for primary nervous pathways (Singer et al 1979). Adhesion to the structures the growth cone contacts also plays a role. Molecular dissection of the role of adhesion in growth cone navigation has led to the identification of several general classes of cell surface molecule. Among these are the *immunoglobulin superfamily*, which includes *neural cell adhesion molecule* (N-CAM) and *L1*, the *cadherins*, including *N-cadherin*, and the *integrin family*. The latter is a group of cell surface receptors that are molecular heterodimers which recognize and bind to components of the extracellular matrix, such as fibronectin, laminin and collagen. Thus, both cell-cell and cell-matrix interactions may be involved in axonal pathfinding. Evidence for the role of these molecules has been obtained from culture experiments in which substrata are coated with these molecules, from their immunolocalization in vivo, from perturbation experiments in which antibodies, enzymes or other agents are infused into embryos and axonal trajectories mapped, and studies of invertebrates mutant for various of these molecules. Retinal axons navigate to the optic tectum along pathways lined with the extracellular matrix molecule laminin (Cohen et al 1987), for example, while their fasciculation behaviour in the nerve, and defasciculation once they reach the tectum is governed to some extent by their levels and molecular expression of N-CAM (Schlosshauer et al 1984). Unravelling these processes is likely to prove complicated, however, for while molecules such as laminin may provide a permissive substratum for axon outgrowth, others such as tenascin exhibit growth-promoting or inhibitory properties (Spring et al 1989). In addition, the cell surface integrins comprise many different subtypes, which may recognize more than one extracellular matrix ligand.

The possible role played by *contact inhibition* in developmental processes has also been investigated in recent years. Culture experiments have shown that when chick peripheral sensory neurons and central neurons (retinal ganglion cells) are confronted, their motility is inhibited and they exhibit growth cone collapse (Kapfhammer et al 1986). This reduction or loss of growth cone structure may be analogous to contact paralysis of non-neuronal cells. Purification of fractions from embryonic brain has identified the protein *collapsin* as the active agent. The correct topographic arrangement of terminals in the optic tectum may also rely on such a mechanism. Axons from temporal retina grow on membranes prepared from rostral tectum (their appropriate target) but fail to grow on those prepared from caudal tectum (their inappropriate target; Walter et al 1987). In addition, motor axons that grow from the spinal cord preferentially through the rostral portion of the sclerotome appear to avoid the caudal sclerotome due to contact inhibition. Glycoprotein fractions isolated from somites cause growth cone

collapse that resembles that mediated by collapsin (Davies et al 1990^[1]).

Although many factors have been identified as having positive or negative effects on axonal growth and navigation, a crucial question is how direction might be specified. The attraction of chemotropism as a mechanism is that direction is provided by the gradient of chemotropic substance diffusing away from the target. However, there has also been considerable interest in the idea that gradients of adhesion might provide directional cues to growth cones—*haptotaxis*. In culture, growth cones will grow preferentially along paths of greater adhesiveness (Letourneau 1975^[2]), but there is little firm evidence to suggest that such choices are made in vivo. In the moth wing epidermis, axon extension across distal or proximal regions that exhibit adhesive differences were proposed to reflect haptotactic phenomena (Nardi & Kafatos 1976^[3]). In reality very few axons may actually be pioneers, the majority growing on glial cells and pre-established axon fascicles, so that only the first few must respond to directional cues. At present, the consensus of opinion as to the mechanisms of axon guidance is that cues are multiple, with a different combination prevailing at different points in the pathway.

Once growth cones have arrived in their general target area, there is the additional problem of forming terminals and synapses. In recent years, much emphasis has been placed on the idea that patterns of connectivity depend on the death of inappropriate cells. Cell death during development coincides with the period of synaptogenesis, and present opinion favours the idea that it occurs due to failure of neurons to acquire a sufficient amount of a trophic factor (reviewed in Oppenheim et al 1992^[4]). Coincident firing of neighbouring neurons that have found the appropriate target region might be involved in eliciting release of factor(s), thus reinforcing correct connections. Such mechanisms may explain the numerical correspondence between neurons in a motor pool and the muscle fibres innervated (Tennyson 1969^[5]). If an axon fails to make the correct contacts, its parent soma atrophies and dies, probably as a result of toxic materials liberated within it. *Programmed cell death (apoptosis)* is a definable process with a morphology that is distinct from necrosis, and occurs in neurons (reviewed in Oppenheim 1991^[6]). Activation of a specific set of genes appears to be responsible for mediating cell death, while other genes can negate the apoptotic programme. Transfection of chick with the gene *Bcl-2* blocks cell death following withdrawal of trophic factors from some sensory neurons and sympathetic neurons (Garcia et al 1992^[7]; Allsopp et al 1993^[8]). On a subtler level, pruning of collaterals may give rise to mature neuronal architecture. The projections of pyramidal neurons from the motor and visual cortex, for example, start out with similar architecture, and the mature repertoire of targets is produced by pruning of collaterals leading to loss of projections to some targets (see p. 230^[9]).

The final growth of dendritic trees is also influenced by patterns of afferent connections and their activity; if deprived of afferents experimentally, dendrites fail to develop fully and, after a critical period, may become permanently affected even if functional inputs are restored, e.g. in the visual systems of young animals visually deprived (Blakemore 1974^[10], 1991^[11]). Metabolic factors also affect the final branching patterns of dendrites, for example, thyroid deficiency in perinatal rats results in a small size and restricted branching of cortical neurons. This may be analogous to the mental retardation of cretinism (Eayrs 1955^[12]).

Once established, dendritic trees appear remarkably stable and partial deafferentation affects only dendritic spines or similar small details. If cells lose all afferent connections or are totally deprived of sensory input (see Guillery 1974^[1]), atrophy of much of the dendritic tree and even the whole neuron ensues, though different regions of the nervous system vary quantitatively in their response to such *anterograde transneuronal degeneration* (p. 920^[2]). Similar effects occur in *retrograde transneuronal degeneration*. As development proceeds plasticity is lost and soon after birth a neuron is a stable structure with a reduced rate of growth.

The Role of Trophic Factors




The existence of maintenance factors in the nervous system was postulated in the case of the developing tetrapod limbs, from which such factors were thought to be conveyed to the CNS where they were capable of influencing the turnover of neuroblasts, i.e. the balance between the rate of proliferation and degeneration. Extirpation of the limb buds of chick embryos led to a massive reduction in numbers of motor and sensory neuroblasts implying that they were dependent on peripheral structures for their survival. These ideas were given substance by the isolation and characterization of nerve growth factor (NGF) (Levi-Montalcini 1950^[3], 1952^[4], 1960^[5], 1967^[6]; Cohen 1958^[7]; Cohen & Levi-Montalcini 1956^[8]; Levi-Montalcini & Chen 1971^[9]) from tissue and tumour extracts and snake venom. These authors demonstrated both in vivo and in vitro influences of NGF on the form and extent of nerve cell growth. Antibodies to NGF cause the death of neuronal subsets at times when they have reached their targets, and added NGF rescues neurons that would otherwise die. NGF is synthesized by various peripheral target organs of the nervous system (Levi-Montalcini & Angeletti 1968^[10]) from which it is taken into the nerve endings and transported back to the neuronal somata. It is necessary for the survival of many types of neuroblasts during early development and their axon and dendritic growth, and promotes the synthesis of neurotransmitters and enzymes. Since the discovery of NGF, several other trophic factors have been identified. Brain derived neurotrophic factor was purified, and subsequently neurotrophin-3 (NT-3) and NT- 4/5 were identified by molecular cloning. Neurotrophins exert their survival effects selectively on particular subsets of neurons, though some neurons can be supported by more than one neurotrophin (reviewed in Thoenen 1991^[11]). Extensive culture experiments have indicated that NGF is specific to *sensory ganglion cells* from the neural crest, *sympathetic postganglionic neurons* and *basal forebrain cholinergic neurons*. BDNF promotes the survival of *retinal ganglion cells*, the *motor neurons*, *sensory proprioceptive* and *placode-derived neurons*, such as those of the nodose ganglion that are unresponsive to NGF. NT-3 has effects on motor neurons, and both placode and neural crest derived sensory neurons. Other growth factors found to influence the growth and survival of neural cells include the *fibroblast growth factors* (FGFs) and *ciliary neurotrophic factor* (CNTF), all of which are unrelated in sequence to the NGF family. Members of the FGF family support the survival of embryonic neuroblasts from many regions of the CNS. CNTF may control the proliferation and differentiation of sympathetic ganglion cells and astrocytes.

Each of the neurotrophins binds specifically to certain receptors on the cell surface. The receptor termed *p75* binds all the neurotrophins with similar affinity. By contrast, members of the family of receptor *tyrosine kinases* (Trks) bind with higher affinity and display binding preferences for

particular neurotrophins. The presence of a Trk receptor seems to be required for p75 function. So far three Trk receptors have been identified. TrkA is the receptor for NGF, TrkB binds BDNF and NT-4/5, and TrkC binds NT-3. Possibilities of other interactions between factors and Trk receptors, such as an effect of NT-3 on TrkA and TrkB, have also been raised. Much progress in understanding the role of neurotrophins *in vivo* has recently been made by generating mice with null mutations of the Trk receptors and of the neurotrophins themselves. Homozygous TrkA mutant mice showed loss of small diameter temperature and pain afferents, and of sympathetic neurons (Smeyne et al 1994^[1]), corresponding to the neuronal subsets normally supported by NGF in culture. The exception were the cholinergic neurons in the basal forebrain, which were supported by NGF in culture, but whose numbers were unaffected in the null mutants. TrkB mutant mice showed loss of neurons in some sensory ganglia, particularly those receiving a placodal contribution, such as the vestibular ganglion. Motor neuron numbers were also reduced (Klein et al 1993^[2]). This implies an effect on the neuronal subsets normally dependent on BDNF. Interestingly, in BDNF mutant mice motor neuron numbers are approximately normal, implying that trophic effects on motor neurons may be mediated via binding of NT-4 to TrkB (Ernfors et al 1993^[3]). The results of the TrkC knockout showed a loss of neurons from dorsal root ganglia, particularly the large diameter afferents mediating proprioception (Klein et al 1994^[4]). This was consistent with observations of the survival-promoting effects of NT-3 on proprioceptive neurons *in vitro*, but it is not clear whether the other neuronal types supported by NT-3 in culture are also lost in the mutant.

Many uncertainties surround interpretations of these knockout experiments and their bearing on the role of these factors *in vivo*, for while TrkB and TrkC transcripts are widely distributed throughout the CNS, no gross defects centrally were seen in the TrkB and TrkC mutant mice. Although the neurotrophins were first envisaged as having their action via production by target organs, the situation has become considerably more complicated. Like NGF, BDNF and NT-3 are present in peripheral tissues, but unlike NGF, they are also expressed by motor and sensory neurons, raising the possibility of autocrine actions. In the future, new growth factors are likely to be identified and the full spectrum of their effects remains to be discovered. Nevertheless, the survival-promoting effects of BDNF and CNTF on motor neurons has led to the initiation of clinical trials with these molecules on patients with amyotrophic lateral sclerosis (neurotrophins reviewed in Loughlin & Fallon 1992^[5]; Lindsay et al 1994^[6]).


In addition to receiving trophic support from other tissues, nervous tissue also influences the metabolism of other tissues. This is true for many cell types, but neuronal effects are, however, perhaps more marked and far reaching. The most obvious example is the mutual dependence of motor neurons and muscles. If, during development, a nerve fails to connect with its muscle, both degenerate. But if the innervation of slow (red) or fast (white) skeletal muscles, each with peculiar functional properties, is exchanged, the muscles change structure and properties in accordance with innervation, indicating that nerve determines muscle type and not vice versa (Buller 1970^[7], and see p. 904^[8]). However, in this case the type of muscle is apparently determined chiefly by the firing pattern of the efferent nerve fibre, rather than by any release of trophic chemical (Lømo & Westgaard 1974^[9]). Trophic influences are clearest in lower vertebrates, in which a denervated limb rudiment fails to complete its development in the absence of nerve-mediated influences (Hamburger 1968^[10]). In higher vertebrates axons have







trophic influence on the dendritic trees they innervate (p. 957) , and on sensory structures. For example, the taste buds degenerate after denervation and regenerate only if the sensory nerves are present. Conversely, if auditory sensory cells are eliminated, the auditory neurons begin to degenerate and many eventually die, suggesting that in this case the trophic influence is the reverse of the usual situation. One recent example of such an interaction comes from mice mutant for the TrkC neurotrophin receptor, which show selective loss of 1a afferents to muscle spindles. In these mice the muscles are devoid of muscle spindles, presumably due to their deafferentation (Klein et al 1994). In addition to these presumably trophic effects, development is replete with examples of the nervous system influencing other tissues, such as the inductive influence of the optic vesicle on adjacent ectoderm resulting in the formation of a lens vesicle and the reciprocal influences of the developing lens and perioptic mesenchyme on the differentiation of the optic cup (p. 259).

Peripheral Nervous System


Autonomic Nervous System

The autonomic nervous system is, apart from the motor axons arising from the CNS, formed by the neural crest. This system includes the *sympathetic* and *parasympathetic neurons* in the peripheral *ganglia* with their accompanying glia, the *enteric nervous system* and glia, and the *adrenal medulla*.

In the trunk at neurulation, neural crest cells migrate from the neural epithelium to lie transitorily on the fused neural tube. Thereafter crest cells migrate laterally then ventrally to their respective destinations (3.110). Within the head the neural crest cells migrate prior to neural fusion producing a vast mesenchymal population as well as autonomic neurons.





The work of Le Douarin and co-workers has provided many data on the contribution of the neural crest in the development of the autonomic nervous system (Le Douarin 1982; Le Douarin 1992). Much of the work has been carried out on chick-quail chimeras (see p. 220) using histochemical markers to map the distribution of different categories of autonomic neurons. The results of this work have provided a map of four major regions of neural crest cell distribution to the autonomic nervous system, including *cranial*, *vagal*, *trunk* and *lumbosacral* crest. The cranial neural crest gives rise to the *cranial parasympathetic ganglia*, whereas the vagal neural crest gives rise to the *thoracic parasympathetic ganglia*. The trunk neural crest gives rise to the *sympathetic ganglia*, mainly the paravertebral ganglia, and *adrenomedullary cells*. This category is often referred to as being of the cells of the *sympathoadrenal lineage* (Patterson 1990; Carnahan & Patterson 1991; Scott-Duff et al 1991.

Neurons of the enteric nervous system are described as arising from the *vagal crest*, i.e. neural crest derived from *somite levels 1–7* (also see below), and *sacral crest*, caudal to the 28th somite. At all of these levels the crest cells are also differentiating into glial-like support cells alongside


the neurons (3.111 ).

Parasympathetic Ganglia

Cranial Neural Crest

In mammalian embryos, crest cells migrate from the region of the mesencephalon and rhombencephalon during the 4–10 somite stages (Nichols 1987 ; Chan & Tam 1988 ). By the 11 somite stage the wave of migration is greatest in the more caudal parts of the latter regions, with most in the rhombencephalon. In avian embryos, crest migration occurs at the level of the developing posterior mesencephalon and preotic myelencephalon during the 4 somite stage onwards (D'Amico-Martel & Noden 1983 ). The *ciliary ganglion* is formed by neural crest cells from the caudal third of the mesencephalon and the rostral metencephalon which migrate along or close to the ophthalmic branch of the trigeminal nerve. It may be reinforced by cells migrating from the nucleus of the oculomotor nerve along which a few scattered cells are always demonstrable in postnatal life. The *pterygopalatine ganglion* derives from preotic myelencephalic crest cells and may receive contributions from the ganglia of the trigeminal and facial nerves. The *otic* and *submandibular* ganglia also derive from myelencephalic neural crest and may have contributions from the glossopharyngeal and facial cranial nerves respectively (2.29C .



Vagal Neural Crest

Neural crest cells from the region located between the midotic placode and the caudal limit of somite 3 have been termed *cardiac neural crest*; they migrate through pharyngeal arches 3, 4, and 6 where they provide, inter alia, support for the embryonic aortic arch arteries, cells of the aorticopulmonary septum and truncus, and cells which differentiate into the neural anlage of the *parasympathetic ganglia* of the heart. Sensory innervation of the heart is from the inferior ganglion of the vagus, which is derived from the nodose placodes (see p. 224 ). Neural crest cells migrating from the level of somites 1–7 (see above) are collectively termed vagal neural crest; they have been demonstrated to migrate to the gut along with sacral neural crest.

Sacral Parasympathetic Ganglia

These have attracted little recent attention; most studies have examined the development of the enteric nerves (see below).

Sympathetic Ganglia

There is much variation in the timing of migration and differentiation of neural crest cells in the trunk region in different species, though the cells commencing migration first migrate furthest distally. In mammalian species, neural crest cells migrate ventrally during the 3–5 somite stage, to penetrate the underlying somites (Erikson et al 1989 ; Serbedzija et al 1990 ). Within a few hours the cells migrate further ventrally to the region of the future *paravertebral* and

prevertebral plexa, notably forming the *sympathetic chain* of ganglia, as well as the major ganglia around the ventral visceral branches of the abdominal aorta (Serbedzija et al 1990^[1]). In avian embryos crest cells migrate preferentially through the rostral half of the somites to appear dorsolateral to the aorta. They spread paravertebrally as well as making contributions to the suprarenal medulla and paravertebral plexa (Le Douarin & Teillet 1974^[2]). The normal segmentation and size of the primary sympathetic ganglia in chicks have been found to depend on alternation of rostrocaudal characteristics of somites such that this positioning may regulate the direction of crest cells towards dorsal root ganglia and sympathetic ganglia (Goldstein & Kalcheim 1991^[3]). The local environment has been shown to be important in determining crest cell fate; after selective deletions of specific crest regions sympathetic ganglia still formed normally in the absence of dorsal root ganglia, thus implying that crest-derived precursors were uncommitted until receiving a stop signal (Scott 1984^[4]). Interestingly, in the quail embryo after crest cells have differentiated and formed dorsal root ganglia, there are dormant autonomic neuronal precursors in the dorsal root ganglia capable of differentiation into adrenergic cells. Furthermore, the differentiation of such cells is dependent on cell–cell interaction.

Pre- and Postganglionic Cell Differentiation and Growth

It has been shown that there is cell specific recognition of postganglionic neurons and the growth cones of sympathetic preganglionic neurons they meet during their growth, and this may be important in guidance to their appropriate targets (Moorman & Hume 1990^[5]). The position of postganglionic neurons, and the exit point from the spinal cord of preganglionic neurons may influence the types of synaptic connections made, and the affinity for particular postganglionic neurons (Lichtmann et al 1979^[6]; Purves et al 1981^[7]). When a postganglionic neuroblast is in place it grows axons (and dendrites) and synaptogenesis follows (Rubin 1985a^[8], b^[9], c^[10]). In mammalian embryos the earliest axonal outgrowths from the superior cervical ganglion occur at about stage 15 (Rubin 1985a^[8]) and although the axon is the first cell process to appear, the position of the neurons does not apparently influence the appearance of the cell processes. There have been many studies of the innervation of peripheral targets (in rats) by sympathetic postganglionic nerves during postnatal life which have relied upon biochemical and immunohistological demonstration of neurotransmitter substances present; (Cochard et al 1979^[11]; Teitelman et al 1979^[12]). In this animal model, at an equivalent to human stage 17 (41 days), there will be many preganglionic axons in the superior cervical ganglion and they can influence electrical activity of postganglionic neurons (Rubin 1985c^[10]). By the time of birth, substantial numbers of functional synapses can be found in the superior cervical ganglion (Rubin 1985c^[10]), some 10% of the adult number (Smolen and Raisman 1980^[13]). Findings in the parasympathetic ganglia are somewhat similar. In the chick ciliary ganglion, for instance, postganglionic axons have been reported to grow in a similar manner towards their targets (Landsmesser et al 1974a^[14]), and there is a significant reduction in the numbers of ganglion cells during development, though synapses form on all cells present prior to death (Landsmesser et al 1974b^[15]), thus exhibiting a general phenomenon in ganglion maturation.

Phenotypic Expression of Differentiating Autonomic Neurons

The elegant transplantation studies performed by Le Douarin's group have demonstrated that the

local environment is the major factor which controls the appropriate differentiation of the presumptive autonomic ganglion neurons. The identity of the factors responsible for subsequent adrenergic, cholinergic or peptidergic phenotypic expression has yet to be elucidated, though fibronectin (Loring et al 1982; Sieber-Blum et al 1981) and basal lamina components molecules (Maxwell & Forbes 1987) have been suggested to initiate adrenergic phenotypic expression at the expense of melanocyte numbers. Cholinergic characteristics are acquired relatively early as shown by studies of premigratory neural crest cells (Le Blanc et al 1990) and the appropriate phenotypic expression may be promoted by cholinergic differentiation factor (Fukada 1985; Yamamori et al 1989) and ciliary neurotrophic factor (Lin et al 1989; Stöckli et al 1989). Schotzinger and Landis (1988) have demonstrated the development of a cholinergic phenotype by noradrenergic sympathetic neurons after innervation of a novel cholinergic target (sweat glands) in vivo. Neuropeptides are expressed by autonomic neurons in vitro and may be stimulated by various target tissue factors in sympathetic (Wong & Kessler 1987) and parasympathetic (Coulombe & Nishi 1991) neurons. Indeed, García-Ararras et al (1986) point out that some neuropeptides are expressed more intensely during early stages of ganglion formation. At what stage coexistence of neuropeptides in synaptic vesicles of preganglionic nerves (e.g. Mitchell & Stauber 1993) and the chemical coding of pre- and postganglionic neurons occurs as in adult ganglion neurons (e.g. Mitchell et al 1993), is unknown.

A number of gene products are expressed on migrating neural crest cells, including the murine homeobox gene cluster *Hox-a* (Toth et al 1987; Galliot et al 1989), detected on dorsal root ganglion cells rather than sympathetic ganglia, *Hox-b* (Wilkinson et al 1989a; Holland & Hogan 1988) expressed in the nodose ganglia and in parts of the enteric nervous system, *Hox-c* (Breier et al 1988) in sympathetic ganglia, and (Robert et al 1989) on cephalic neural crest. Other gene products expressed by crest cells include the cellular oncogene c-ets 1 (Vandenbunder et al 1989) and the zinc finger gene *Krox-20* (Wilkinson et al 1989a) which is found on differentiating dorsal root and cranial ganglion cells. Two genes whose malexpression leads to abnormal development of neural crest-derived nervous system elements are *Hox a-4* which is associated with abnormal development of the enteric nervous system (Wolgemuth et al 1989), and *Hox a-1* which results in malformations of hindbrain development (Lufkin et al 1991; Chisaka et al 1992), and consequent derangements of cranial nerves.

Enteric Nervous System

The enteric nervous system is different from the other components of the autonomic nervous system as, unlike the sympathetic and parasympathetic ganglia, the enteric nervous system can mediate reflex activity **independently** of control by the brain and spinal cord (Gershon 1987). The number of enteric neurons which develop is believed to be of the same magnitude as the number of neurons in the spinal cord (Furness & Costa 1980). The number of preganglionic fibres which supply the intestine, and therefore modulate the enteric neurons, are much fewer. This discrepancy led Langley (1921) to postulate that most of the enteric neurons receive no direct input from the CNS at all. (The position of neural crest contribution to the enteric nervous system is shown in 3.111.)

It is worth noting at this point that enteric nerves have more in common with central nerves than peripheral nerves. Enteric nerves do **not** have the collagenous coats of extraenteric peripheral nerves, and, as in the CNS, there is no endoneurium within the enteric plexuses; rather the cells are supported by *glia* which closely resemble astrocytes and contain glial fibrillary acidic protein (GFAP). Although Schwann cells surrounding unmyelinated nerves also contain detectable amounts of GFAP, enteric glia contain more. Interestingly the enteric glia do not produce a surrounding basal lamina as do Schwann cells. Gershon (1987^[4]) reviews this field.

Chimeric experimentation has demonstrated that quail donor crest taken from axial levels, which do not normally supply the gut, and transplanted into the vagal regions of chick hosts produces an embryo with entirely quail enteric nerves. Thus the premigratory neural crest cells are not pre-patterned for specific axial levels; rather they attain their axial value as they leave the neuraxis. Once within the gut wall there is a regionally specific pattern of enteric ganglia formation (Gabella 1981^[4]) suggested to be controlled by the local splanchnopleuric mesenchyme.

The most caudal derivatives of neural crest cells, from the lumbosacral region, form components of the pelvic plexus after migrating through the somites towards the level of the colon, rectum and cloaca. The cells come to lie initially within the developing mesentery and then transiently between the layers of the differentiating muscularis externa before finally, and later, forming a more substantial intramural plexus characteristic of the adult enteric nervous system. Of the neural crest cells that colonize the bowel, some of the foregut have been reported to acquire the ability to migrate outwards and colonize the developing pancreas, as the characteristic population of autonomic neurons (Kirchgeßner et al 1992^[4]).

Hirschsprung's disease is suggested to result from a failure of neural crest cells to colonize the gut wall appropriately. The condition is characterized by a dilated segment of colon proximally and lack of peristalsis in the segment distal to the dilatation. Infants with Hirschsprung's disease show delay in the passage of meconium, constipation, vomiting and abdominal distension. Gershon (1987^[4]) has investigated a similar condition in the *ls/ls* mouse which develops congenital megacolon secondarily to aganglionosis of the terminal portion of the large bowel. The non-peristaltic portion of gut contains thick nerves in the adventitia, muscularis externa and submucosa but the number of cells in the submucosal plexus is reduced compared to normal mice. There are numerous aberrantly located ganglia outside the gut connected by axons to the nerves within the gut. Both the submucosal nerves and the aberrant ganglia have the characteristics of non-enteric neurons. Immunocytochemical studies of the enteric basal lamina have shown an increase in the amount of laminin, type IV collagen and heparan sulphate in the aganglionic gut. In normal mice laminin and type IV collagen are found beneath the mucosal and serosal epithelia and around the blood vessels. In the *ls/ls* mouse there is a broad zone of expression of these proteins around the entire outer gut mesenchyme, specifically of the aganglionic portion of bowel. Gershon (1987^[4]) suggests that the overabundance of basal laminal components may prevent the neural crest cells from penetrating the gut wall; their new position outside the gut does not confer on them the environmental stimuli for enteric nerve differentiation so non-enteric development occurs in local ganglia adjacent to the gut.

In humans Hirschsprung's disease is often seen associated with other defects of neural crest development, e.g. Waardenburg type II syndrome which includes deafness and facial clefts with megacolon.

Chromaffin Cells

Chromaffin cells are derived from the neural crest and found at numerous sites throughout the body. As well as the classic chromaffin cells of the *suprarenal medulla*, others in this group include the *bronchial neuroepithelial cells*, *dispersed epithelial endocrine cells* of the gut (formerly known as *argentaaffin cells*), *carotid body cells*, and the *paraganglia*.

The sympathetic ganglia, suprarenal (adrenal) medulla and chromaffin cells are all derived from the cells of the *sympathoadrenal lineage* (Patterson 1990; Carnahan & Patterson 1991; Scott-Duff et al 1991). In the suprarenal medulla these cells differentiate into a number of types consisting of *small* and *intermediate-sized neuroblasts* or *sympathoblasts* and larger, initially rounded *phaeochromocytoblasts*. Molenaar et al (1990) have described the development of chromaffin cells in the suprarenal medulla in human fetuses aged 6–34 weeks. Using various markers two morphological cell types could be identified: large cells with pale nuclei from about 9 weeks, and clusters of small cells, appearing slightly later, at about 14 weeks. Their observations on the differential distribution of markers within these two cellular populations led them to conclude that the large cells were the progenitors of the chromaffin cells in the suprarenal medulla whilst the smaller cells were neuroblasts. The intermediate-sized neuroblasts differentiate into the typical *multipolar postganglionic sympathetic neurons* (which secrete noradrenaline at their terminals) of classic autonomic neuroanatomy. The smaller neuroblasts have been equated with the *small intensely fluorescent* (SIF) cells, types I and II. Both have been shown (at least in some species and sites) to be dopamine-storing and secreting cells. It is postulated that type I function as true interneurons, synapsing with the principal postganglionic neurons. Type II are thought to operate as local neuroendocrine cells, secreting dopamine into the ganglionic microcirculation. Both types of SIF cells probably modulate the principal preganglionic/postganglionic synaptic transmission. The large cells differentiate into masses of columnar or polyhedral *phaeochromocytes* (*classic chromaffin cells*) which secrete either adrenaline or noradrenaline. These cell masses are termed *paraganglia* and may be situated near, on the surface of, or embedded in the capsules of the ganglia of the sympathetic chain, or in some of the large autonomic plexuses. The largest members of the latter are the *para-aortic bodies* which lie along the sides of the abdominal aorta in relation to the inferior mesenteric artery. During childhood the para-aortic bodies and the paraganglia of the sympathetic chain partly degenerate and can no longer be isolated by gross dissection, but even in the adult chromaffin tissue can still be recognized microscopically in these various sites. It may be noted here that both the phaeochromocytes and the SIF cells, using a wider and more recent classification, are regarded as chromaffin; they belong to the amine precursor uptake and decarboxylation (APUD) series of cells and are paraneuronal in nature.

A pivotal role for glucocorticoids in the development of the chromaffin cell lineage has been emphasized by Hofmann et al (1989) and later by Michelsohn and Anderson (1992) who

found that the development of the chromaffin phenotype involves two sequential, glucocorticoid-dependent events, and both appear to be mediated by the type II glucocorticoid receptor.

Enhancement of chromaffin cell process outgrowth, however, may be facilitated by various cell adhesion and extracellular matrix molecules (Poltorak et al 1990^[5]). Cell adhesion molecules play a major role in determination of tissue architecture during histogenesis of the suprarenal gland (Leon et al 1992^[6]). Two neural cell adhesion molecules N-CAM and L1 have been found to be expressed in the adult rat suprarenal gland. The expression of catecholamine synthesizing enzymes tyrosine hydroxylase and phenylethanolamine N-methyltransferase was correlated with the adhesion molecule expression. Groups of L1 and N-CAM positive cells were found to display different phenotypic expression of catecholamine synthesizing enzymes. Environmental factors also play an important role in the development of the physical and biochemical characteristics of chromaffin cells (Mizrachi et al 1990^[7]). Co-culture of human pheochromocytoma cells with adrenal endothelial cells induced the tumour cells to acquire physical and biochemical characteristics of chromaffin cells, exhibiting similar organization as seen in vivo. The rapid transient increase in the change in the state of the proto-oncogene c-fos expression suggests that the mechanism(s) inducing the change in the state of the differentiation of tumour cells in co-culture with endothelial cells may be distinct from that described for the differentiation of chromaffin cells by glucocorticoids.

The differentiation of chromaffin cells appears not to be immutably fixed. Jousselin-Hosaja et al (1993^[8]) found that mouse suprarenal chromaffin cells can transform to neuron-like cholinergic phenotypes after being grafted into the hippocampus. Transdifferentiation of chromaffin cells to the neuron-like phenotype was also found to be induced after nerve growth factor application (de la Torre et al 1993^[9]).

The timing of the appearance of neurotransmitters and neuropeptides in chromaffin cells is not comprehensively established. In the avian sympathoadrenal system, however, 5HT-like immunoreactivity was found to be transiently expressed by chromaffin cells very early in development (E5–E8), disappearing almost entirely at more advanced embryonic stages (E10–E19) and in posthatched chicks where only a population of cells similar to mammalian SIF cells express immunoreactivity to 5HT (García-Arraras & Martinez 1990^[10]). In relation to the development of catecholamine-synthesizing enzymes, in rat embryos, Anderson et al (1991^[11]) noted that sympathoadrenal precursors are first identifiable in primordial sympathetic ganglia at E11.5 when they express tyrosine hydroxylase. At this stage, the progenitors also coexpress neuronal markers, but also a series of chromaffin cell markers called SA 1–5. The observation of double labelled cells is consistent with the hypothesis that these cells represent a common progenitor to sympathetic neurons and suprarenal chromaffin cells. After E11.5, sympathetic ganglia no longer express chromaffin markers. Neuropeptides are also expressed by developing chromaffin cells. García-Arraras et al (1992^[12]) have demonstrated the expression and development of neuropeptide Y (NPY)-like immunoreactivity in chromaffin cells of the chicken. They also found that NPY is coexpressed with somatostatin and serotonin. The expression of neuropeptides such as leucine enkephalin may be regulated by glucocorticoids and preganglionic nerves in suprarenal chromaffin cells (Henion & Landis 1990^[13]). These factors, therefore,

participate in the generation of the mature neurochemical phenotypes present in the suprarenal medulla, both during development and in adults.

Suprarenal Glands

Each suprarenal gland consists of a *cortex* derived from *coelomic epithelium* and a *medulla* into which *neural crest cells* migrate from somite levels 18–24 migrate (3.110, 111). The cortex is formed during the second month by a proliferation of the coelomic epithelium; cells pass into the underlying mesenchyme between the root of the dorsal mesogastrum and the mesonephros (Keene & Hewer 1927; Crowder 1957). The proliferating tissue extends from the level of the sixth to the twelfth thoracic segments. It is soon disorganized dorsomedially by invasion of neural crest cells which form the medulla and also by the development of venous sinusoids. The latter are joined by capillaries which arise from adjacent mesonephric arteries and penetrate the cortex in a radial manner. When proliferation of the coelomic epithelium ceases the cortex is enveloped ventrally, later dorsally, by a mesenchymal capsule derived from the mesonephros. The subcapsular nests of cortical cells are the rudiment of the *zona glomerulosa*. These nests proliferate cords of cells which pass deeply between the capillaries and sinusoids. The cells in these cords degenerate in an erratic fashion as they pass towards the medulla, becoming granular, eosinophilic and ultimately autolysed. These cords of degenerating cells constitute the *fetal cortex*, which undergoes a rapid degeneration during the first two weeks after birth with marked shrinkage of the gland. The *fascicular* and *reticular* zones of the adult cortex are proliferated from the glomerular zone after birth and are only fully differentiated by about the twelfth year.

Somatic Nerves

Spinal Nerves

Each spinal nerve is connected to the spinal cord by a ventral root and a dorsal root. The fibres of the ventral roots grow out from cell bodies in the anterior and lateral parts of the mantle zone; these pass through the overlying marginal zone and external limiting membrane to enter the *myotomes* of the somites, or penetrate the latter, reaching the adjacent somatopleure, and in both sites ultimately form the α -, β - and γ -efferents. At appropriate levels these are accompanied by the outgrowing axons of preganglionic sympathetic neuroblasts (segments T1–L2), or preganglionic parasympathetic neuroblasts (S2–S4).

The fibres of the dorsal roots extend from the cells of the spinal ganglia. Before the neural groove is closed to form the neural tube, a ridge of neurectodermal cells, the *neural crest* (*ganglion ridge*), appears along the prominent margin of each neural fold. When the folds meet in the median plane the two neural crests fuse into a wedge-shaped mass along the line of closure of the tube. Neural crest cells are produced continuously along the length of the spinal cord, but gangliogenic cells migrate only into the rostral part of each somitic sclerotome where they condense and proliferate to form a bilateral series of oval-shaped *primordial spinal ganglia*. In



addition to negative factors in the caudal sclerotome that deter neural crest cells from entering (see p. 155), the rostral sclerotome has a mitogenic effect on the crest cells that settle within it (Goldstein et al 1990). From the ventral region of each ganglion a small part separates to form *sympatho-chromaffin cells* (p. 236), while the remainder becomes a *definitive* spinal ganglion. The spinal ganglia are arranged symmetrically at the sides of the neural tube and, except in the caudal region, are equal in number to the somites. The cells of the ganglia, like the cells of the mantle zone of the early neural tube, are glial and neuronal precursors. The glial precursors develop into the satellite cells, which become closely applied to the ganglionic nerve cell somata (perikarya), into Schwann cells, and possibly other cells. The neuroblasts, at first round or oval, soon become fusiform, with extremities gradually elongating into central and peripheral processes. The central processes grow into the neural tube, as the fibres of dorsal nerve roots, while the peripheral processes grow ventrolaterally to mingle with the fibres of the ventral root thus forming a *mixed spinal nerve*. As development proceeds the original bipolar form of the cells in the spinal ganglia changes; the two processes become approximated until they ultimately arise from a single stem in a T-shaped manner, to form a unipolar cell (sometimes, less appropriately, termed pseudo-unipolar). The bipolar form is, however, retained in the ganglion of the vestibulocochlear nerve.

The position of the early neural crest as a wedge-shaped mass along the line of tube closure noted above, and the identification of ganglionic cells in various positions in the wall of the early neural tube, and even within the central canal (Humphrey 1944, 1947), is strongly reminiscent of the developmental history of the *Rohon-Beard cells* in fish and amphibia (Rohon 1884; Beard 1896), which are thought to be important in the emergence of primitive locomotor patterns (Hughes 1968). In this regard, other investigators have claimed that in primitive chordates (Cyclostomes and Euselachians) the neural crest develops as an *evagination* of the dorsal region of the dorsolateral lamina of the late neural folds and early neural tube (Conel 1942). For a review of the origin, widespread migration and differentiation of neural crest cells see Weston (1970) Bellairs (1971) Leikola (1976) and page 220.






Cranial Nerves

Cranial nerves may contain motor, sensory or both types of fibres. With the exception of the olfactory and optic nerves, the cranial nerves develop in a manner similar in some respects to components of the spinal nerves. The somata of motor neuroblasts originate within the neuroepithelium, while those of sensory neuroblasts are derived from the neural crest and from ectodermal placodes.









The *motor fibres* of the cranial nerves to striated muscle are the axons of cells in the basal plate of the midbrain and hindbrain that grow outwards to their muscle fibres of distribution. The functional and morphological distinction between the neurons within these various nerves is based on the types of muscle innervated. In the trunk, the motor roots of the spinal nerves all emerge from the spinal cord close to the ventral midline to supply the muscles derived from the somites.

In the head the motor outflow is segregated into two pathways (3.112 , 117 ). *General somatic efferent* neurons exit ventrally in a similar manner to those of the spinal cord, comprising the oculomotor, trochlear, abducens and hypoglossal nerves. Thus nerves III, IV, VI and XII parallel the organization of the somatic motor neurons in the spinal cord. The second motor component, *special branchial efferent*, comprises the accessory nerve and the motor parts of the trigeminal, facial, glossopharyngeal and vagus nerves, whose nerve exit points lie more dorsally than the somatic motor system.

The cranial nerves also contain a third class of efferent neurons, the *general visceral efferent* neurons (parasympathetic preganglionic) travelling in nerves III, VII, IX and X, which leave the hindbrain via the same exit points as the special branchial efferent fibres. All these three categories of motor neurons probably originate from the same region of the basal plate, adjacent to the floor plate. The definitive arrangement of nuclei then arises due to differential migration of neuronal somata. It is not known whether all these cell types share a common precursor within the rhombencephalon, though there is evidence that in the spinal cord somatic motor and preganglionic autonomic neurons are lineally related.

These motor neuron types have been thus designated according to the types of muscles or structures innervated. General somatic efferent nerves supply striated muscle now known to be derived from the *cranial (occipital) somites* and *prechordal mesenchyme*. Myogenic cells from the ventrolateral edge of the epithelial plate of occipital somites give rise to the intrinsic muscles of the tongue, while the prechordal mesenchyme gives rise to the extrinsic ocular muscles (see p. 274 ). Special branchial efferent nerves supply the *striated muscles developing within the pharyngeal (branchial) arches* (3.148 ). Chimeric experiments have shown that myogenic cells in the pharyngeal arches are derived solely from unsegmented paraxial mesenchyme, rostral to the somites, which migrates into the arch primordia (Noden 1983a ; Couly et al 1992 ; see p. 275 ). Thus **all the voluntary muscles of the head originate from axial (prechordal) or paraxial mesenchyme** rendering the distinction between somatic efferent supply and branchial efferent supply somewhat artificial. However, the obviously special nature of the arch musculature, its patterning by the neural crest cells, its particularly rich innervation for both voluntary and reflex activity and the different origins from the basal plate of the branchial efferent nerves compared to the somatic efferent nerves, make the retention of a distinction between the two of value.

General visceral efferent neurons (parasympathetic preganglionic) innervate glands of the head, the *sphincter pupillae* and *ciliary muscles*, and the *thoracic* and *abdominal viscera*.

The *cranial sensory ganglia* are derived in part from the neural crest, and in part from cells of the ectodermal placodes (3.103 , 112 , 148 ; see p. 222 ). Generally, neurons distal to the brain derive from placodes while proximal ones derive from the neural crest (D'Amico-Martel & Noden 1980 , 3.148 ); supporting cells of all sensory ganglia arise from the neural crest (Noden 1978 ; D'Amico-Martel 1981 ). The most rostral sensory ganglion, the *trigeminal* (V) comprises both neural crest and placode-derived neurons that mediate *general somatic afferent* functions. In the case of more caudal cranial nerves (VII, IX and X) the same

applies, but the two cell populations form **separate** ganglia in the case of each nerve. Analogous with the trigeminal, the proximal series of ganglia is neural crest derived (forming the proximal ganglion of VII, the *superior* ganglion of IX and the *jugular* ganglion of X) while the distal series derives from placodal cells (forming the *geniculate* ganglion of VII, the *petrosal* ganglion of IX and the *nodose* ganglion of X). These ganglia contain neurons that mediate *special*, *general visceral* and *somatic afferent* functions. The VIIth nerve has a *vestibular ganglion* containing both crest and placodal cells and an acoustic ganglion from placodal neurons only; it conveys special somatic afferents.

Both neurons and supporting cells of the *cranial autonomic ganglia* in the head and the trunk originate from neural crest cells (Weston 1971📖; Le Douarin & Teillet 1974📖; see p. 223📖). Caudal to the ganglion of the vagal nerve the occipital region of the neural crest is concerned with the 'ganglia' of the accessory and hypoglossal nerves. Rudimentary ganglion cells may occur along the hypoglossal nerve in the human embryo; they undergo regression later. Ganglion cells are also found on the developing spinal root of the accessory nerve and these are believed to persist in the adult. The central processes of the cells of these various ganglia, where they persist, form some sensory roots of the cranial nerves and enter the alar lamina of the hindbrain; their peripheral processes join the efferent components of the nerve to be distributed to the various tissues innervated. Some incoming fibres from the facial, glossopharyngeal and vagal nerves collect to form an oval bundle, the *tractus solitarius*, on the lateral aspect of the myelencephalon. This bundle is the homologue of the oval bundle of the spinal cord, but in the hindbrain it becomes more deeply placed by the overgrowth, folding and subsequent fusion of tissue derived from the rhombic lip on the external aspect of the bundle.

Central Nervous System








Spinal Cord

In the future spinal cord the median *roof plate* (*dorsal lamina*) and *floor plate* (*ventral lamina*) of the neural tube do not participate in the cellular proliferation affecting the lateral walls and hence remain thin. Their cells contribute largely to the formation of the ependyma.


The neuroblasts of the *lateral* walls of the tube are large and at first round or oval (*apolar*). Soon they develop processes at opposite poles, becoming *bipolar neuroblasts*. One process is, however, withdrawn and the neuroblast becomes *unipolar*, although this is not invariably so in the case of the spinal cord. Further differentiation leads to the development of dendritic processes and they become typical multipolar neurons. In the developing cord they occur in small clusters representing clones of neurons. The development of a longitudinal *sulcus limitans* on each side of the central canal of the cord divides the ventricular and mantle zones in each lateral wall into a *basal* (*ventrolateral*) *lamina* or *plate* and an *alar* (*dorsolateral*) *lamina* or *plate* (3.113👁️). This separation indicates a fundamental functional difference, for neural precursors in the basal lamina include the motor cells of the anterior (ventral) and lateral grey columns, while those of the alar lamina exclusively form 'interneurons' (those possessing both short and



long axons), some of which receive the terminals of primary sensory neurons. Caudally the central canal of the cord exhibits a fusiform dilatation, the *terminal ventricle*.

Anterior (Ventral) Grey Column

The cells of the ventricular zone are closely packed at this stage and arranged in radial columns (3.94 ). For experimental studies of radial migration patterns consult Berquist (1932 , 1968 ), also bibliographies in Rakic (1981 , 1982 ). Their disposition may be partly determined by contact guidance along the earliest *radial array* of glial fibres that traverse the **full thickness** of the early neuroepithelium. The cells of the intermediate (mantle) zone are more loosely scattered, and they increase in number at first in the region of the basal lamina. This enlargement outlines the *anterior (ventral) column* of the grey matter and causes a ventral projection on each side of the median plane, the floor plate remaining at the bottom of the shallow groove so produced. As growth proceeds these enlargements, further increased by the development of the *anterior funiculi* (regions of axons passing to and from the brain), encroach on the groove until it becomes converted into the slit-like anterior median fissure of the adult spinal cord (3.113 ). The axons of some of the neuroblasts in the anterior grey column traverse the marginal zone and emerge as bundles on the anterolateral aspect of the spinal cord as the *ventral spinal nerve rootlets*. These constitute, eventually, both the α -efferents which establish motor end plates on extrafusal striated muscle fibres and the γ -efferents which innervate the contractile polar regions of the intrafusal muscle fibres of the muscle spindles (p. 971 ). (The histogenesis of β -efferents is completely uncharted.)

Lateral Grey Column

In the thoracic and upper lumbar regions some intermediate (mantle) zone neuroblasts in the dorsal part of the basal plate outline a *lateral column*. Their axons join the emerging ventral nerve roots and pass as preganglionic fibres to the ganglia of the sympathetic trunk or related ganglia, the majority eventually myelinating to form *white rami communicantes*. The fibres constituting the rami establish synapses with the autonomic ganglionic neurons, and the axons of some of the latter proceed as postganglionic fibres to innervate smooth muscle cells, adipose tissue or glandular cells. Some of the preganglionic sympathetic efferent axons pass to the cells of the suprarenal medulla. The innervation of other 'chromaffin' tissues is less certain (but see the carotid body, p. 971 ). Similarly an autonomic lateral column is also laid down in the midsacral region and gives origin to the preganglionic para-sympathetic fibres of the pelvic splanchnic nerves.

The **changes** in cell number, position and density as seen in a longitudinal section of the chick spinal cord, based on investigations of Hamburger (1952 ), are illustrated in 3.114 . The anterior region of each basal plate forms at first a continuous column of cells throughout the length of the developing cord. In many forms this soon develops into two columns (on each side)—one medially placed, concerned with innervation of axial musculature, and a lateral one innervating the limbs. At limb levels the latter column enlarges enormously but regresses at other levels.

Thus the development of the cord involves an interplay between a number of fundamental processes which vary in prominence at different times and at different levels—cell *proliferation*, *migration*, followed either by progressive cell *growth* and *differentiation* or, in complete contrast, by cell *degeneration* and *death*. In the example quoted, cell proliferation persists as a prominent feature at the levels concerned with limb innervation, whilst at thoracic levels a *dorsomedial* migration of neuroblasts occurs to lay the foundation of the visceral efferent column. Further, save at limb levels, massive cell degenerations occur in the lateral 'motor' columns, whereas the medial columns, which innervate axial musculature, persist throughout the cord. The phenomenon of cell death and removal on a large scale, balanced against local proliferation and migration rates, has only been recognized relatively recently as a fundamental feature in many morphogenetic situations.

Thus, it has been shown, some ventrolateral laminal neuroblasts differentiate into the ventral horn neurons from which α -, β - and γ -efferent fibres arise, and these are accompanied at thoracic, upper lumbar and midsacral levels by preganglionic autonomic efferents from neuroblasts of the developing lateral horn. However, additionally, numerous interneurons develop in both these situations (including the well-studied Renshaw cells), but it is uncertain how many of these differentiate directly from ventrolateral lamina (basal plate) neuroblasts and how many migrate to their final positions from the dorsolateral lamina (alar plate).

In the human embryo, the definitive grouping of the ventral column cells, which characterizes the mature cord, occurs early, and by the fourteenth week (80 mm) all the major groups can be recognized (Romanes 1942, 1946, 1953, 1964).




As the anterior and lateral grey columns assume their final form the germinal cells in the ventral part of the ventricular zone gradually cease to proliferate and the layer becomes reduced in thickness until it ultimately forms the single-layered ependyma which lines the ventral part of the central canal of the spinal cord.

Posterior (Dorsal) Grey Column

The posterior (dorsal) column is somewhat late in its development and, as a result, its ventricular zone is for a time much thicker in the dorsolateral lamina (alar plate) than it is in the ventrolateral lamina (basal plate) (3.94).


While the columns of grey matter are being defined, the dorsal region of the central canal becomes narrow and slit-like and its walls come into apposition and fuse with each other (3.113). In this way the central canal becomes relatively reduced in size and somewhat triangular in outline.

About the end of the fourth week advancing axonal sprouts invade the marginal zone. The first to develop are those destined to become short *intersegmental* fibres from the neuroblasts in the intermediate (mantle) zone, and also fibres of *dorsal roots* of spinal nerves which pass into the

spinal cord from neuroblasts of the early spinal ganglia. The earlier dorsal root fibres that invade the dorsal marginal zone stem from **small** dorsal root ganglionic neuroblasts. By the sixth week the latter form a well-defined *oval bundle* near the peripheral part of the dorsolateral lamina (3.94 , 3.113 ); this bundle increases in size and, spreading towards the median plane, forms the *primitive posterior funiculus*; its constituent fibres are destined to be of fine calibre. Later, fibres derived from new populations of **large** dorsal root ganglionic neuroblasts join the dorsal root to become fibres of much larger calibre. As the posterior funiculi increase in thickness, their medial surfaces come into contact separated only by the *posterior medial septum*, which is ependymal in origin, neuroglial in nature. A more detailed analysis of the temporal sequence of modifications of the dorsal lamina (roof plate), posterior medial septum, and the lateral displacement of the *primitive posterior funiculus* with the later development of the fasciculus gracilis, based on a study of sectioned human embryos of 6–10 weeks and dissections of fetuses up to the end of the fourth month, has been provided by Hughes (1976 ). He proposed that the displaced primitive posterior funiculus may form the basis of the dorsolateral tract or fasciculus (of Lissauer), and also correlated the sequence, siting and calibre of the entrant dorsal root fibres with the changing size-distribution of the somata of the dorsal root ganglionic neuroblasts.

At about the third month long intersegmental fibres begin to appear and at about the fifth month corticospinal fibres. All nerve fibres are at first without myelin sheaths and different groups **commence** to develop sheaths at different times, e.g. the ventral and dorsal nerve roots about the fifth month, the corticospinal fibres after the ninth month. In peripheral nerves the myelin is formed by Schwann cells (derived from neural crest cells); in the CNS by oligodendrocytes (which develop from the ventricular zone of the neural tube). Myelination, of course, persists until overall growth of the CNS and PNS has ceased. Thus, in many sites, slow growth continues for long periods, even into the postpubertal years.

The cervical and lumbar enlargements first appear simultaneously with the development of their respective limb buds.

In early embryonic life the spinal cord occupies the **entire length** of the vertebral canal and the spinal nerves pass at right angles to the cord. After the embryo has attained a length of 30 mm the vertebral column begins to grow more rapidly than the spinal cord, the caudal end of which gradually becomes more cranial in the vertebral canal. Most of this relative rostral migration occurs during the first half of intrauterine life. By the twenty-fifth week the terminal ventricle of the spinal cord has altered in level from the second coccygeal vertebra to the third lumbar, a distance of nine segments, and there remain but two segments before the adult position is reached (Streeter 1919 ). As the change in level begins rostrally, the caudal end of the terminal ventricle, which has become adherent to the overlying ectoderm, remains in situ and the walls of the intermediate part of the ventricle and its covering pia mater become drawn out to form a delicate filament, the *filum terminale*. The separated portion of the terminal ventricle persists for a time but it usually disappears before birth. It does, however, occasionally give rise to congenital cysts in the neighbourhood of the coccyx. In the definitive state, the upper cervical spinal nerves retain their position roughly at right angles to the cord; proceeding caudally,

however, the nerve roots lengthen and are progressively more oblique.

Brain

A summary list of the derivatives of the cerebral vesicles from caudal to rostral is given below:

Rhombencephalon (or Hindbrain)

1. Myelencephalon	Medulla oblongata Caudal part of the 4th ventricle Inferior cerebellar peduncles
2. Metencephalon	Pons Cerebellum Middle part of the 4th ventricle Middle cerebellar peduncles
3. Isthmus rhombencephali	Anterior medullary velum Superior cerebellar peduncles Rostral part of the 4th ventricle

Mesencephalon (or midbrain)

Cerebral peduncles
Tegmentum
Tectum
Aqueduct

Prosencephalon (or Forebrain)

1. Diencephalon	Thalamus Metathalamus Subthalamus Epithalamus Caudal part of the hypothalamus Caudal part of the 3rd ventricle
2. Telencephalon	Rostral part of the hypothalamus Rostral part of the 3rd ventricle Central hemispheres Lateral ventricles

Rhombencephalon

By the time the midbrain flexure appears, the hindbrain exceeds in length the combined extent of the other two brain vesicles. Rostrally it exhibits a constriction, the *isthmus rhombencephali* (3.96B), best viewed from the dorsal aspect. Ventrally the hindbrain is separated from the dorsal wall of the primitive pharynx only by the notochord, the two dorsal aortae and a small amount of mesenchyme; on each side it is closely related to the dorsal ends of the pharyngeal arches (3.141).

The pontine flexure appears to 'stretch' the thin, epithelial roof plate which becomes widened, the greatest increase in width corresponding to the region of maximum convexity, so that the outline of the roof plate becomes rhomboidal. By the same change the lateral walls become separated, particularly dorsally, and the cavity of the hindbrain, subsequently the fourth ventricle, becomes flattened and somewhat triangular on cross-section. The pontine flexure becomes increasingly acute until, at the end of the second month, the laminae of its cranial (metencephalic) and caudal (myelencephalic) slopes are opposed to each other (3.116C) and, at the same time, the lateral angles of the cavity extend to form the lateral recesses of the fourth ventricle.

About the end of the fourth week, when the pontine flexure is first discernible, a series of six transverse *rhombic grooves* appears in the ventrolateral lamina (basal plate) of the hindbrain. Between the grooves, the intervening masses of neural tissue are termed *rhombomeres* (see p. 219). These are closely associated with the pattern of the underlying motor nuclei of certain of the cranial nerves. The distribution of motor nuclei has been determined for avia and for rodents, but it is not known whether this pattern is conserved in humans. Rhombomere 1 contains the trochlear nucleus, rhombomeres 1, 2 and 3 contain the trigeminal nucleus, rhombomeres 4 and 5 the facial nucleus, rhombomeres 5 and 6 the abducens nuclei, rhombomeres 6 and 7 the glossopharyngeal nucleus, and rhombomeres 7 and 8 the vagus, accessory and hypoglossal nerves. Rhombomeric segmentation represents the ground plan of development in this region of the brainstem and is pivotal for the development of regional identity (see p. 229 and see also 3.148). With further morphogenesis, however, the obvious constrictions of the rhombomere boundaries disappear, and the medulla once again assumes a smooth contour. The differentiation of the lateral walls of the hindbrain into basal (ventrolateral) and alar (dorsolateral) plates has a similar significance to the corresponding differentiation in the lateral wall of the spinal cord (p. 238) and ventricular, mantle and marginal zones are formed in the same way.

Cells of the Basal Plate (Ventrolateral Lamina)

These are often, in elementary accounts, simply termed 'motor' (but see below); they form **three** elongated, but interrupted, **columns** positioned ventrally and dorsally with an intermediate column between (3.115).






(1) The most *ventral column* is continuous with the anterior grey column of the spinal cord and will supply muscles considered 'myotomic' in origin. It is represented in the caudal part of the hindbrain by the hypoglossal nucleus, and it reappears at a higher level as the nuclei of the abducent, trochlear and oculomotor nerves, which are *somatic efferent nuclei*.

(2) The *intermediate column* is represented in the upper part of the spinal cord and caudal brainstem (medulla oblongata and pons) and is for the supply of branchial (pharyngeal) and postbranchial musculature. It is interrupted also, but the caudal brainstem part, which gives fibres to the ninth, tenth and eleventh cranial nerves, forms the elongated *nucleus ambiguus*. The latter continues into the cervical spinal cord as the origin of the spinal accessory nerve. At higher levels parts of this column give origin to the motor nuclei of the facial and trigeminal nerves. These three nuclei are termed *branchial (special visceral) efferent nuclei*.

(3) The most dorsal column of the basal plate (represented in the spinal cord by the lateral grey column) innervates viscera. It is interrupted also, its large caudal part forming some of the *dorsal nucleus* of the vagus and its cranial part the *salivatory nucleus*. These are termed *general visceral (general splanchnic) efferent nuclei* and their neurons give rise to preganglionic, parasympathetic nerve fibres.

It should be noted here that the neurons of the basal plate and their three columnar derivatives are only 'motor' in the sense that **some** of their number form either α , β or γ motor neurons, or preganglionic parasympathetic neurons. The remainder, which greatly outnumber the former, differentiate into functionally related interneurons and, in some loci, neuroendocrine cells.

Cell Columns of the Alar Plate (Dorsolateral Lamina)

These are also interrupted and give rise to *general visceral (general splanchnic) afferent*, *special visceral (special splanchnic) afferent*, *general somatic afferent* and *special somatic afferent* nuclei (their relative positions, in simplified transverse section, are shown in 3.115 ). The general visceral afferent column is represented by a part of the dorsal nucleus of the vagus (see also p. 1251 ), the special visceral afferent column by the nucleus of the tractus solitarius, the general somatic afferent column by the afferent nuclei of the trigeminal nerve (Brown 1974 ) and the special somatic afferent column by the nuclei of the vestibulocochlear nerve. (Again it should be noted here that the relatively simple functional independence of these afferent columns implied by the foregoing classification is, in the main, an aid to elementary learning. The emergent neurobiological mechanisms are in fact much more complex and less well understood). Although they tend to retain their primitive positions, some of these nuclei are later displaced by differential growth patterns and by the appearance and growth of neighbouring fibre tracts, and possibly by active migration. It has been suggested that a neuron tends to remain as near as possible to its predominant source of stimulation and that when the possibility of separation arises, owing to the development of neighbouring structures, it will migrate in the direction from which the greatest density of stimuli come. This phenomenon was termed neurobiotaxis (Kappers 1921 , 1934 ). Cells can migrate in this way only by lengthening of their axons, which therefore trace the route taken by the cells on their transit. The curious courses of the fibres

arising from the facial nucleus (p. 1243) and nucleus ambiguus have been held to illustrate this phenomenon. In the 10-mm embryo the facial nucleus lies in the floor of the fourth ventricle, occupying the position of the special visceral efferent column, and it is placed at a higher level than the abducent nucleus. As growth proceeds the facial nucleus migrates at first caudally and dorsally, relative to the sixth nerve nucleus, and then ventrally to reach its adult position. As it migrates, the axons to which its somata give rise elongate and their subsequent course is assumed to map out the pathway along which the facial nucleus has travelled. Similarly the nucleus ambiguus arises initially immediately deep to the ventricular floor, but in the adult it is more deeply placed and its efferent fibres first pass dorsally and medially before curving laterally to emerge at the surface of the medulla oblongata. Neurobiotaxis has been relatively well-documented for the case of branchial (special visceral) efferent and general visceral efferent neurons in the brainstem, whose somata are translocated dorsolaterally from the motor column towards their exit point and targets. Certainly the absence of placodal neurons which contribute to the trigeminal nerve results in the remaining crest derived neurons failing to undergo their normal migrations from medial to lateral positions in the floor of the metencephalon (Noden 1991).

Myelencephalon

The caudal slope of the embryonic hindbrain constitutes the myelencephalon, which develops into the medulla oblongata. The nuclei of the ninth, tenth, eleventh and twelfth cranial nerves develop in the situations already indicated and afferent fibres from the ganglia of the ninth and tenth nerves form an oval marginal bundle in the region overlying the alar (dorsolateral) lamina. The dorsal edge of this lamina throughout the rhombencephalon gives attachment to the thin expanded roof plate and is termed the *rhombic lip*. (The *inferior rhombic lip* is confined to the myelencephalon; the *superior rhombic lip* to the metencephalon.) As the walls of the rhombencephalon spread outwards, the rhombic lip protrudes as a lateral edge which becomes folded over the adjoining area. The rhombic lip may later become adherent to this area, and its cells migrate actively into the marginal zone of the basal plate. In this way the oval bundle which forms the *tractus solitarius* becomes buried. Alar plate cells which migrate from the rhombic lip are believed to give rise to the olivary and arcuate nuclei and the scattered grey matter of the nuclei pontis. While this migration is in progress the thin floor plate is invaded by fibres which cross the median plane (accompanied by neurons that cluster in and near this plane), and it becomes thickened to form the *median raphe*. Some of the migrating cells from the rhombic lip in this region do not reach the basal plate and form an oblique ridge across the dorsolateral aspect of the inferior cerebellar peduncle: the *corpus pontobulbare* (*nucleus of the circumolivary bundle*).

The lower (caudal half) part of the myelencephalon takes no part in the formation of the fourth ventricle and, in its development, it closely resembles the spinal cord. The nuclei, *gracilis* and *cuneatus*, and some reticular nuclei, are derived from the alar plate, and their efferent arcuate fibres and interspersed neurons play a large part in the formation of the median raphe.

At about the fourth month the descending *corticospinal fibres* invade the ventral part of the medulla oblongata to initiate the pyramids whilst dorsally, ascending fibres from the spinal cord,

with olivocerebellar and parolivocerebellar fibres, external arcuate fibres, together with two-way reticulocerebellar and vestibulocerebellar interconnections, form the inferior cerebellar peduncle. (The reticular nuclei of the lower medulla probably have a dual origin from both basal and alar plates.)




Metencephalon

The rostral slope of the embryonic hindbrain is the metencephalon, from which both cerebellum and pons develop. Before formation of the pontine flexure the dorsolateral laminae of the metencephalon are parallel with one another. Subsequent to its formation the roof plate of the hindbrain becomes rhomboidal and the dorsal laminae of the metencephalon lie obliquely, being close at the cranial end of the fourth ventricle but widely separated at the level of its lateral angles. Accentuation of the flexure approximates the cranial angle of the ventricle to the caudal, and the alar plates of the metencephalon now lie almost horizontally.




Caudal to the developing cerebellum the roof of the fourth ventricle remains epithelial, covering an approximately triangular zone from the lateral angles of the rhomboid fossa to the median obex. Over this region nervous tissue fails to develop and vascular pia mater is closely applied to the subjacent ependyma. At each lateral angle and in the midline caudally the membranes break through forming the lateral and median apertures of the roof of the fourth ventricle. Subsequently, these are the principal routes by which cerebrospinal fluid, produced in the ventricles, escapes into the subarachnoid space. The vascular *pia mater* (*tela choroidea*), in an inverted V formation cranial to the apertures, invaginates the ependyma to form vascular fringes—the vertical and horizontal parts of the choroid plexuses of the fourth ventricle.

Cerebellum


The cerebellum consists of a cortex within which are buried a series of deep nuclei. This organization of the cerebellar cortex is similar to that of the cerebral cortex, except that the latter has six layers, while the former has only three. These are the *outer molecular layer*, the *Purkinje layer*, containing Purkinje cells which are the only output neurons of the cortex, and the *inner granular layer*.


Two rounded swellings develop which at first project partly into the ventricle (3.116B,C ) , forming the rudimentary cerebellar hemispheres. The most cranial part of the roof of the metencephalon originally separates the two swellings, but it becomes invaded by cells, which form the rudiments of the vermis. These cells were regarded as derivatives of both basal and alar plates (Baxter 1953 ). At a later stage, *extroversion of the cerebellum* occurs, with reduction of its *intraventricular* projection and an increasing dorsal *extraventricular prominence*. The cerebellum now consists of a bilobar (dumb-bell shaped) swelling stretched across the rostral part of the fourth ventricle (3.116 ), continuous rostrally with the anterior medullary velum, formed from the isthmus, and caudally with the epithelial roof of the myelencephalon. With growth a number of transverse grooves appear on the dorsal aspects of the cerebellar rudiment, as the precursors of the numerous fissures which characterize the surface of the mature



cerebellum (3.117 , also p. 243 ).

The *posterolateral fissure*, in its lateral parts, appears first, demarcating the then most caudal area from the rest of the cerebellar rudiment, enabling the *flocculi* to be identified. The lateral parts of this fissure extend medially, meet in the median plane and demarcate the nodule. The *flocculonodular lobes* can now be recognized and are the most caudal part of the cerebellum at this stage, but, owing to the growth of the adjoining areas, they progressively come to occupy the **anterior** part of the *inferior surface* in the adult. They are formed in proximity to the line of attachment of the epithelial roof, i.e. to the rhombic lip (p. 243  and 3.116 , 117 ).

At the end of the third month a transverse sulcus appears on the rostral slope of the cerebellar rudiment and deepens to form the *fissura prima*, which cuts into the vermis and both hemispheres, separating the most cranial region to form the anterior lobe.

During the same period two short transverse grooves appear on the inferior vermis; the first is the *fissura secunda*, which demarcates the uvula, and the second the *prepyramidal fissure*, demarcating the pyramid (3.116B ). The whole cerebellum now grows dorsally and the caudo-inferior aspects of the *hemispheres* expand much more than the inferior vermis, which therefore becomes buried at the bottom of a deep hollow—the *vallecula*. Meanwhile numerous additional fissures develop, which are approximately parallel to, and intervene between, the foregoing. They result in a relatively vast increase in surface area of the cerebellum but their precise positions and systematic names have limited functional or morphological significance. The most extensive of these develops into the *horizontal fissure*.

In many mammals a part of the hemisphere immediately rostral to the posterolateral fissure becomes defined as an entity; in some it forms a very prominent part of the cerebellum, termed the *paraflocculus*, but the relationship is purely topographical and, in contrast to the flocculus, the paraflocculus receives afferent connections mainly, but not entirely, from the cerebral cortex. It is uncertain whether any homologue of the paraflocculus exists in the human cerebellum or whether, as has been proposed, it is represented by some small patches of grey matter which are frequently present on the inferior surface of the middle cerebellar peduncle (Larsell 1947 .

The emergence of arrays of principal fissures ('fissuration') and folia ('foliation') has been studied experimentally by a number of workers (Sievers et al 1981 ; Allen et al 1981 ). Proliferation of the external granular layer and its expansion relative to the white matter beneath appears to have a role in cortical folding. Other factors include outgrowth and differentiation of the fibres constituting the central white core, the Bergmann glia, the external granular ventricular zone, the pial/glial basal lamina and the pial fibroblasts. Selective experimental alteration of any of these elements alters or obliterates the folial pattern. (For these tissue components see below.)

Mammalian Cerebellar Histogenesis

Mammalian cerebellar histogenesis has been more completely described, both during normal ontogeny and after experimental intervention, than in any other part of the nervous system, due

in large measure to its precisely ordered geometry and highly distinctive cell types. The connectivity, synaptology and electrophysiology of the latter have also been intensively studied, and some knowledge of these aspects is a prerequisite of any account of histogenesis (Section 8). Particularly valuable in histogenetic investigations have been nuclear labelling with tritiated thymidine, the analysis of genetic variants and the changes following surgical deafferentation, exposure to biochemically active agents, X-irradiation and virus diseases. Most studies have been confined to mice and rats, and whilst it seems probable that **qualitatively** similar cell migrations and contacts occur, **quantitative** findings and **ontogenetic timings** cannot, of course, be extrapolated to the human cerebellum. Only the briefest introduction can be entertained in this volume, and the interested reader should enter the literature by consulting such key references as Miale and Sidman (1961), Fujita (1963), Fujita et al (1966), Kornhuber et al (1966, 1968), Mugnaini (1970), Eccles (1970), Hamori (1972), Altman (1972 and 1972a, c), Eccles (1973), Swarz (1976), Swarz and del Cerro (1975, 1977), Berry (1982); Berry et al (1980a, b, c, d).

The early cerebellar rudiment consists of a pseudostratified epithelium showing interkinetic migration (p. 229) which soon develops the three basic zones—ventricular, mantle, and marginal—as elsewhere in the neural tube. The rudiment of the cerebellum originates from cells of the superior rhombic lip and adjacent dorsal part of the alar plate of the metencephalon and mesencephalon. Studies on avia have shown a hitherto unrecognized contribution to the cerebellum of the *mesencephalon*, due to the fact that at the isthmus, the dorsal part of the mesencephalon is displaced caudally and comes to overlies the myelencephalon (Hallonet et al 1990). The mesencephalon and metencephalon give rise to different cell populations within the cerebellum. The genetic control of cerebellar development is being investigated, and may depend upon the gene *Wnt-1*, whose expression domain forms an annulus, rostral to the isthmus; *Wnt-1* mutant mice have cerebellar defects (Thomas & Capecchi 1990).

The histogenesis of the cerebellum is complicated by the existence of **two** germinal zones, the first a ventricular zone lying beneath the developing cerebellar plate, the *internal germinal layer*, and the second coming to lie superficial to the mantle zone. The latter, termed the *external germinal layer*, is derived from cells migrating exclusively from the *metencephalic rhombic lip*. Initially, progenitor cells continue proliferating in the ventricular zone. Slightly later, spreading lateromedially from the rhombic lips to meet centrally in a subpial position (i.e. the most superficial part of the marginal zone), similarly uncommitted germinal cells proliferate forming the external germinal layer. Both layers continue *proliferative mitoses*, giving clonal progeny, until they are several cells thick. This *proliferative phase* gradually diminishes with the onset of the *phase of neurogenesis*, and eventually overlaps and merges into a *phase of gliogenesis*. The generation of cell types in the cerebellum follows a precisely ordered time sequence. Specific neuronal varieties that characterize the deep cerebellar nuclei and cortex migrate to their definitive positions, develop axons and synaptic contacts, and mature (Altman & Bayer 1978; Berry et al 1980d). Similarly, as neurogenesis wanes, remaining germinal cells continue to give generations of committed glioblasts which although less well documented than the neuroblasts, also migrate to and mature in their definitive positions. The exception to this is the specialized, radially disposed *Bergmann glioblasts*, however, which are amongst the **first** cells to be committed in the early neuroepithelium and may provide contact guidance pathways for

neuronal migration. The fate of each germinal layer will now briefly be reviewed.

Within the mantle layer, neurons differentiate in two strata, the superficial stratum giving rise to Purkinje cells of the cerebellar cortex and the deeper stratum giving rise to the neurons of the roof nuclei. Initially, *primitive Purkinje neuroblasts* and *primitive nuclear neuroblasts* emerge in approximately equal numbers, but whether this follows a series of symmetrical or asymmetrical mitoses is unknown. The nuclear neuroblasts remain embedded in the developing future white matter adjacent to the roof of the rostral part of the fourth ventricle. The main mass of nuclear neuroblasts then slowly subdivides into the primordial *fastigial*, *emboliform*, *globose* and *dentate deep cerebellar nuclei*, and the individual neuroblasts differentiate as either small intranuclear interneurons or the larger projection neurons. The axons of the latter invade the early cerebellar peduncles and pursue complex paths to their multiple destinations.

The Purkinje neuroblasts, in contrast, **migrate** superficially towards their definitive position in the expanding cortex, where they slowly mature into their highly characteristic form of somata and dendritic trees. As they migrate, the terminals of one process—the future axon—remain adjacent to, and ultimately in synaptic contact with, the nuclear neuroblasts, the remainder of the axon elongating as it 'trails' behind the advancing soma. The mature and developing Purkinje cell has been a favourite object for **quantitative** cytological and ultrastructural studies, both during normal ontogeny and after such experimental manipulations as suppression of granule cell (and therefore parallel fibre) development, or after prevention of climbing fibre growth (Hamori 1972 and p. 1036). The maturation of normal rat cerebellar cortical neurons has been described in normal development (Altman 1972b), and after experimental manipulation (Berry et al 1978, 1980a, b, c, d); Rakic 1981, 1982). When the formation of nuclear and Purkinje neurons has proceeded for some time, a number of the remaining cells of the ventricular zone give rise to generations of *Golgi neurons*, the small neurons of the roof nuclei and possibly some glia. These cells also migrate superficially to gradually occupy, and mature in, their definitive position and morphology.

The external germinal (also termed granular) layer, formed by migration of cells from the rhombic lip, is the origin of the *granule cells*, *basket cells*, *stellate cells* and probably some *glial cells*. These cells migrate centripetally to come to lie at varying depths within the developing cerebellum. Granule cells migrate through the molecular and Purkinje cell layers to form the *internal granular layer*. Basket and stellate cells, however, migrate only as far as the molecular layer. Basket cells migrate deeply to meet, and ultimately synapse with, the somata and axons of the ascending Purkinje neuroblasts, their axons passing transversely in the primordial cerebellar folia. Following more prolonged proliferation of the external granular layer vast numbers of granule cell neuroblasts are produced. Ramón y Cajal first described the migration of granule cells, based on Golgi preparations. He realized that the different morphologies of granule cells within different cortical layers represented a dynamic sequence. In the deeper part of the external granular layer the cells are bipolar, with two processes oriented parallel to the long axes of the folia, in a similar manner to the mature parallel fibres of the granule cells, each of which contacts many Purkinje cells. Cells at deeper levels possessed a third process oriented orthogonally, down which cells had apparently migrated into the molecular layer. The apparent mystery of this stereotyped vertical migration, despite obstructions, was solved by the discovery that external

granule cells migrate inwards along the long radial processes of specialized glial cells— the Bergmann glia (Mugnaini & Forstrønen 1967^[1]). The general significance of this mechanism in neural development was recognized by Rakic (1971a^[2], b^[3]), who demonstrated the presence of radial glial cells in all parts of the primate brain. He considered that the migrating soma 'trailed' behind it an elongated axon which subsequently bifurcated to form the parallel fibre axons, whilst dividing axons passing in advance of the soma met the mossy afferents to form cerebellar glomeruli. The translocation of neuronal somata along glial processes has been studied extensively in culture, where neurons from the cerebellum or the cortex are equally able to migrate on glia derived from either region (reviewed in Hatten 1990^[4]). There appears to be a reciprocal dependence between neurons and glial cells, with the latter dependent on the neuron to arrest its division and promote its differentiation. A number of molecules may be involved in the migration, but antibodies against N-cadherin and integrin can disrupt the process. Mice mutant for the *weaver* (*wv*) gene develop with small cerebelli, due to loss of granule cells. Granule cells seem to be the primary site of action of *wv*, since *wv* mutant granule cells cannot migrate on wild-type glial cells (Hatten et al 1986^[5]); the gene encodes a membrane-associated ligand that induces neuronal differentiation (Gao et al 1992^[6]). The final generations of progenitor cells from the external germinal layer, relatively sparse and brief, occur whilst granule cell production is at its height; their progeny merely migrate locally and differentiate into outer stellate cells.



The origin of the cerebellar glial cells remains much more problematical and has been the subject of dispute for over a century. The view advanced by Obersteiner (1883) and Schaper (1897^[7]) was that the various macroglial cell varieties (Bergmann cells, astrocytes and oligodendrocytes) stemmed from final generations of germinal cells of both the internal and external germinal layers; this has received more recent experimental support (Fujita et al 1966^[8]; Fujita 1967^[9]; Meller et al 1969^[10]; Privat 1975^[11]). The second view proposed by Athias (1897^[12]) and Ramón y Cajal (1911^[13]) held that the macroglia were formed exclusively from the internal ventricular zone, and that the progeny of the external layer were solely the three varieties of neuroblast: this suggestion has been supported by the labelling studies of Swarz and del Cerro (1977^[14]). The consensus at the moment seems to be that both zones give rise to glia. But while the ventricular zone is responsible for the production of the early Bergmann glia, and of glial cells of the internal granular layer, glia of the molecular layer originate from the external granular layer. The microglial elements are exogenous, invading the cerebellar rudiment from the surrounding vasculature.






The remainder of the metencephalon becomes the pons, but little is known of the individual stages in the transformation. Ventricular, mantle and marginal zones are formed in the usual way, and the nuclei of the trigeminal, abducens and facial nerves develop in the mantle layer. It is possible that the grey matter of the formatio reticularis is derived from the basal plate and that of the nuclei pontis from the alar plate by the active migration of cells from the rhombic lip. However, about the fourth month the pons is invaded by corticopontine, corticobulbar and corticospinal fibres, becomes proportionately thicker, and takes on its adult appearance.

The region of the *isthmus rhombencephali* undergoes a series of changes notoriously difficult to interpret. As a result, however, the greater part of the region apparently becomes absorbed into

the caudal end of the midbrain, only the roof plate, in which the anterior medullary velum is formed, and the dorsal parts of the alar plate, which become invaded by converging fibres of the superior cerebellar peduncles, remaining as recognizable derivatives in the adult. Note that originally the decussation of the trochlear nerves was **caudal** to the isthmus, but as the growth changes occur it is displaced in a rostral direction until it reaches its adult position.

Mesencephalon

The mesencephalon or midbrain, derived from the intermediate primary cerebral vesicle, persists for a time as a thin-walled tube enclosing a cavity of some size, separated from that of the prosencephalon by a slight constriction and from the rhombencephalon by the isthmus rhombencephali. Later, its cavity becomes relatively reduced in diameter, and in the adult brain it forms the *cerebral aqueduct*. The basal (ventrolateral) plate of the midbrain increases in thickness to form the *cerebral peduncles*, which are at first of small size, but enlarge rapidly after the fourth month, when their numerous fibre tracts begin to appear in the marginal zone (p. 1035  et seq). The neuroblasts of the basal plate give origin to the nuclei of the oculomotor nerve and some grey masses of the tegmentum, while the nucleus of the trochlear nerve remains in the region of the isthmus rhombencephali. The trigeminal mesencephalic nucleus originates from midbrain neural crest (Narayanan & Narayanan 1978 ). The cells of the dorsal part of the alar (dorsolateral) plates proliferate and invade the roof plate, which therefore thickens and is later divided into corpora bigemina by a median groove. Caudally this groove becomes a median ridge, which persists in the adult as the frenulum veli. The corpora bigemina are later subdivided into the *superior* and *inferior colliculi* by a transverse furrow. The *red nucleus*, *substantia nigra* and *reticular nuclei* of the midbrain *tegmentum* may first be defined at the end of the third month. Their origins are probably mixed from neuroblasts of both basal and alar plates.

The detailed histogenesis of the tectum and its main derivatives, the colliculi, will not be followed here, but in general the principles outlined for the cerebellar cortex (p. 243 ), the palaeopallium and neopallium (p. 253 ) also apply to this region. There exists a high degree of geometric order in the developing retinotectal projection (the equivalent of the retinogeniculate projection), and also a precise somatotopy in tectospinal projection. These facts, coupled with the ability of the fish and amphibian central nervous tracts to **regenerate** after severance, have led the retinotectal pathways to become classical sites for experimentation. Much interest has centred on two problems: how retinal axons reach the tectum, and how correct topographic connections are made. Many signals have been invoked to explain retinal ganglion axon guidance to the tectum. These may be preformed pathways, physical features, such as a pattern of holes and spaces at the optic nerve head (Silver & Sidman 1980 ), adhesive molecules in the pathway, such as neural cell adhesion molecules (Silver & Rutishauser 1984 ), and pre-existing axon tracts (Easter & Taylor 1989 ). However, the ability of ectopic axons to find the optic tectum when originating from supernumerary transplanted eyes has also suggested some globally-distributed positional information on the neuroepithelial surface, whose molecular identity has not been elucidated. The search for molecules in the specificity of termination sites on the tectum has been slightly more successful. Firstly, the results of perturbation experiments in amphibia led to the elaboration of a powerful theory of 'chemospecificity', in which positional cues on the surfaces of growing axons were envisaged to be shared with the appropriate target

area on the tectum (Sperry 1963¹). The idea of a large number of unique markers was superseded by the idea that graded distributions of a few molecules might play the same role (Fraser 1980²; Gierer 1981³). In vitro, retinal temporal axons are found to prefer to grow on anterior tectal membranes and to avoid posterior membranes, corresponding with their termination site in vivo (Walter et al 1987⁴). Other molecules have been identified that could mediate topographical matching via their distributions, for example the 'Trisler' molecule that is preferentially localized in dorsoposterior compared with ventro-anterior retina (Trisler et al 1981⁵).




Prosencephalon



At an early stage, a transverse section through the forebrain shows the same parts as are displayed in similar sections of the spinal cord and medulla oblongata—thick lateral walls connected by thin floor and roof plates. Moreover, each lateral wall is divided into a dorsal area and a ventral area separated internally by the *hypothalamic sulcus*. This sulcus ends anteriorly at the medial end of the optic stalk (see below); in the fully developed brain, it persists as a slight groove extending from the interventricular foramen to the cerebral aqueduct. It is analogous to, if not the homologue of, the sulcus limitans. The thin roof plate remains epithelial, but invaginated by vascular mesenchyme, the tela choroidea of the choroid plexuses of the third ventricle. Later, the lateral margins of the tela undergo a similar invagination into the medial walls of the cerebral hemispheres (see below). The floor plate thickens, developing the nuclear masses of the hypothalamus and subthalamus.



At a very early period, before the closure of the rostral neuropore (p. 146⁶), two lateral diverticula, the *optic vesicles*, appear, one on each side, about the level of the forebrain; for a time they communicate with its cavity by relatively wide openings. The distal parts of the optic vesicles expand, while the proximal parts become the tubular *optic stalks*. (Their further development is given on pp. 259–261⁷.) The forebrain next grows, its tip curving ventrally, and two further diverticula rapidly expand from it, one on each side. These diverticula are rostromedial to the optic stalks and subsequently form the *cerebral hemispheres*; their cavities are the rudiments of the lateral ventricles; they communicate with the median part of the forebrain cavity by relatively wide openings which ultimately become the interventricular foramina. The anterior limit of the median part of the forebrain consists of a thin sheet, the *lamina terminalis* (3.118A-C⁸), which stretches from the interventricular foramina to the recess at the base of the optic stalks. The anterior part of the forebrain, including the rudiments of the cerebral hemispheres, is the *telencephalon* (*end-brain*), and the posterior part of the *diencephalon* (*between-brain*); both contribute to the formation of the third ventricle, although the latter predominates. The fate of the lamina terminalis is detailed below.

Diencephalon

The diencephalon is broadly divided by the hypothalamic sulcus into the *pars dorsalis diencephali* and *pars ventralis diencephali*; these, however, are composite, each contributing to diverse neural structures. The pars dorsalis develops into the (dorsal) thalamus and metathalamus along the immediate suprasulcal area of its lateral wall, whilst the highest dorsocaudal lateral

wall and roof form the epithalamus. The *thalamus* (3.118A-C ) is first visible as a thickening which involves the anterior part of the dorsal area (Cooper 1950 ). Caudal to the thalamus the lateral and medial geniculate bodies, or *metathalamus*, are recognizable at first as surface depressions on the internal aspect and as elevations on the external aspect of the lateral wall (Cooper 1945 ). With the enlargement of the thalami as smooth ovoid masses, they gradually narrow the wide interval between them into a vertically compressed cavity which forms the greater part of the third ventricle. After a time these medial surfaces may come into contact and become adherent over a variable area, the connection (single or multiple) constituting the *interthalamic adhesion*. The caudal growth of the thalamus excludes the geniculate bodies from the lateral wall of the third ventricle.

At first the lateral aspect of the developing thalamus is separated from the medial aspect of the cerebral hemisphere by a cleft, but with growth the cleft becomes obliterated (3.119 ) as the thalamus fuses with the part of the hemisphere in which the corpus striatum is developing. Later, with the development of the projection fibres (corticofugal and corticopetal) of the neocortex (p. 158 ), the thalamus becomes related to the internal capsule, which intervenes between it and the lateral part of the corpus striatum (lentiform nucleus). Ventral to the hypothalamic sulcus the lateral wall of the diencephalon, in addition to median derivatives of its floor plate, forms a large part of the hypothalamus and subthalamus.

The *epithalamus*, which includes the pineal gland, the posterior and habenular commissures and the trigonum habenulae, develops in association with the caudal part of the roof plate and the adjoining regions of the lateral walls of the diencephalon. At an early period (12–20 mm CR length) the epithalamus in the lateral wall projects into the third ventricle as a smooth ellipsoid mass, larger than the adjacent mass of the (dorsal) thalamus and separated from it by a well-defined *epithalamic sulcus*. In subsequent months growth of the thalamus rapidly overtakes that of the epithalamus; the intervening sulcus is obliterated. Thus, finally, structures of epithalamic origin are topographically relatively diminutive; in recent years, however, there has occurred a burgeoning of interest in, and understanding of, their functional roles (p. 1423 ). The *pineal gland* arises as a hollow outgrowth from the roof plate, immediately adjoining the mesencephalon. Its distal part becomes solid by cellular proliferation, but its proximal stalk remains hollow, containing the pineal recess of the third ventricle. In many reptiles the pineal outgrowth is double. The anterior outgrowth (*parapineal organ*) develops into the pineal or parietal eye (p. 1889 ) while the posterior outgrowth is glandular in character. It is the **posterior** outgrowth which is homologous with the pineal gland in man. The anterior outgrowth also develops in the human embryo but soon disappears entirely.

The *posterior commissure* is formed by fibres which invade the caudal wall of the pineal recess from both sides.

The *nucleus habenulae*, which is the most important constituent of the *trigonum habenulae*, is developed in the lateral wall of the diencephalon and is at first in close relationship with the geniculate bodies, from which it becomes separated by the dorsal growth of the thalamus. The *habenular commissure* develops in the cranial wall of the pineal recess.

The roof plate of the diencephalon, rostral to the pineal gland (and continuing over the median telencephalon) remains thin and epithelial in character and is subsequently invaginated by the choroid plexuses of the third ventricle. Before the development of the corpus callosum and the fornix it lies at the bottom of the longitudinal fissure; between and reaching the two cerebral hemispheres, it extends as far rostrally as the interventricular foramina and lamina terminalis. Here, and elsewhere, choroid plexuses develop by the close apposition of vascular pia mater and ependyma without intervening nervous tissue. With development, the vascular layer is infolded into the ventricular cavity and develops a series of small villous projections, each covered by a cuboidal epithelium derived from the ependyma. The cuboidal cells carry numerous microvilli on their ventricular surfaces whilst basally their plasma membrane becomes complexly folded into the cell. The **early** choroid plexuses secrete a protein-rich cerebrospinal fluid into the ventricular system which may provide a nutritive medium for the primitive epithelial neural tissues. With increasing vascularity of the latter, however, the histochemical reactions of the cuboidal cells and the character of the fluid change to the adult type (Klosovskii 1963). It should also be noted that, in addition to choroid plexus formation, the remaining lining of the third ventricle does **not** simply form generalized ependymal cells. Many regions become highly specialized, developing concentrations of tanycytes or other modified cells, e.g. those of the *subfornical organ*, the *organum vasculosum (intercolumnar tubercle)* of the lamina terminalis, the *subcommissural organ* and those lining the *pineal*, *suprapineal*, and *infundibular recesses* (Knigge et al 1975, Collins and Woollam 1981), collectively termed the *circumventricular organs*.

In addition to its subsulcal lateral walls, the floor of the pars ventralis diencephali takes part in the formation of the *hypothalamus*, including the mamillary bodies, the tuber cinereum and infundibulum of the hypophysis.



The *mamillary bodies* arise as a single thickening, which becomes divided by a median furrow during the third month. Anterior to them the *tuber cinereum* develops as a cellular proliferation which extends forwards as far as the infundibulum. In front of the tuber cinereum the floor of the diencephalon gives origin to a wide-mouthed diverticulum, which grows towards the stomodeal roof and comes into contact with the posterior aspect of a dorsally directed ingrowth from the stomodeum (Rathke's pouch, p. 257). These two diverticula together form the *hypophysis cerebri* (3.128). In the base of the neural outgrowth an extension of the third ventricle persists as the *infundibular recess*. The remaining caudolateral walls and floor of the ventral diencephalon are an extension of the midbrain tegmentum—the *subthalamus*. This forms the rostral limits of the red nucleus and substantia nigra, numerous reticular nuclei and a wealth of interweaving nerve fibre bundles, ascending, descending and oblique, with many origins and destinations.


The optic vesicles, which are described with the development of the eye (p. 259), are derived from the lateral wall of the prosencephalon before the telencephalon can be identified. They are usually regarded as derivatives of the diencephalon and the optic chiasma is often regarded as the boundary between diencephalon and telencephalon.

Telencephalon

The telencephalon (end-brain) consists of two lateral diverticula connected by a median region (the *telencephalon impar*). From the impar develops the anterior part of the cavity of the third ventricle, closed below and in front by the *lamina terminalis*. The lateral diverticula are outpouchings of the lateral walls of the telencephalon, which may correspond to the alar laminae, although this is uncertain; the cavities are the future lateral ventricles, and their walls the primordial nervous tissue of the *cerebral hemispheres*. The roof plate of the median part of the telencephalon remains thin and is, as noted, continuous behind with the roof plate of the diencephalon. In the floor plate and lateral walls of the prosencephalon, **ventral** to the primitive interventricular foramina, the **anterior** parts of the hypothalamus are developed; these include the optic chiasma, optic recess and related nuclei. The chiasma is formed by the meeting and partial decussation of the optic nerves in the ventral part of the lamina terminalis, and from it the optic tracts subsequently grow backwards to end in the diencephalon and midbrain.

Cerebral Hemispheres

The cerebral hemispheres arise as diverticula of the lateral walls of the telencephalon, with which they remain in continuity around the margins of the initially relatively large interventricular foramina, except caudally, where they are continuous with the anterior part of the lateral wall of the diencephalon (3.119A,B ); as growth proceeds the hemisphere enlarges forwards, upwards and backwards and acquires an oval outline, with medial and superolateral walls and a floor. As a result the medial surfaces approach, but are separated from each other by, a vascularized mesenchyme and pia mater that fills the *median longitudinal fissure*. At this stage the floor of the fissure is the epithelial roof plate of the telencephalon, which is directly continuous caudally with the epithelial roof plate of the diencephalon (3.119 ) , as already stated above.



At the early oval stage of hemispheric development, regional names are given in accordance with their future principal derivatives. The rostromedial and ventral *floor* becomes linked with the forming olfactory apparatus and may be termed the primitive *olfactory lobe* (see below and p. 1317 ). The floor (ventral wall, or base) of the larger remainder of the hemisphere forms the anlage of the primitive corpus striatum and amygdaloid complex: hence this, including its associated rim of lateral and medial walls, is the *striate part of the hemisphere*. The rest of the hemisphere, the largest in surface area but initially possessing rather thin walls, medial, lateral, dorsal and caudal, is thus the *suprastrate part of the hemisphere*. The whole of the latter (except the interventricular foramen and its extension, the choroidal fissure) together with the superficial (subpial) zone of the striate part are the areas where histogenesis of named apparent variants of cerebral cortex (or pallium) occur. Further details, and comments on their plethora of terminologies and (often unsatisfactory) classifications, are furnished below.

The rostral end of the oval hemisphere becomes the definitive *frontal pole* but, as the hemisphere expands, its **original** posterior pole moves relatively in a caudoventral and lateral direction, curving thence towards the orbit in association with the growth of the caudate nucleus and



numerous other structures to form the definitive *temporal pole*, and a **new** posterior part becomes evident which persists as the definitive *occipital pole* of the mature brain. The great expansion of the cerebral hemispheres is characteristic of mammals and especially of man, and in their subsequent growth they overlap, successively, the diencephalon and the mesencephalon and then meet the rostral surface of the cerebellum; the temporal lobes embrace the flanks of the brainstem.



The early diverticulum or anlage of the cerebral hemisphere contains initially a simple spheroidal *lateral ventricle* which is continuous with the third ventricle via the interventricular foramen, the rim of the latter being the site of the original evagination. With expansion and the assumption of an oval outline by the hemisphere, the ventricle becomes firstly roughly ellipsoid and then a curved cylinder, convex dorsally. The ends of the cylinder expand towards (but do not reach) the frontal and (temporary) occipital poles—differentiating and thickening neural tissues separate the ventricular cavities and pial surfaces at all points, except along the line of the choroidal fissure (see below). Pronounced changes in ventricular form accompany the emergence of a temporal pole; the original caudal end of the curved cylinder expands within its substance. This temporal extension passes ventrolaterally to encircle its side of the upper brainstem (cf. choroidal fissure below). Finally, from the root of the temporal extension another may develop in the substance of the definitive occipital pole, passing caudomedially; this is quite variable in size, often asymmetrical on the two sides; one or both may be absent. Although a continuous system of cavities, specific parts of the lateral ventricle are now given regional names: the *central part (body)* extends from the interventricular foramen to the level of the posterior edge (splenium) of the corpus callosum. From the body three *cornua (horns)* diverge—**anterior** towards the frontal pole, **posterior** towards the occipital pole and **inferior** towards the temporal pole.

It may be noted that at these early stages of hemispheric development the term pole is preferred, in most instances, to lobe; the latter are defined by specific surface topographical features which will appear over several months, and differential growth patterns persist for a considerable period.

About the fifth week a longitudinal groove appears in the anteromedial part of the floor of each ventricle. This groove deepens and forms a hollow diverticulum continuous with the hemisphere by a short stalk. The diverticulum becomes connected on its ventral or inferior surface to the *olfactory placode* (see p. 278 ) , the cells of which give rise to the afferent axons of its sensory cells. These terminate in the walls of the diverticulum. As the head increases in size the diverticulum grows forwards and, subsequently losing its cavity, becomes converted into the solid *olfactory bulb*. The forward growth of the bulb is accompanied by elongation of its stalk, which forms the *olfactory tract*, and the part of the floor of the hemisphere to which the tract is attached constitutes the *piriform area* (see below). For comments on the accessory olfactory bulb see page 1317 .


The pia mater which covers the epithelial roof of the third ventricle at this stage is itself covered with loosely arranged mesenchyme. In the meshes of this tissue numerous blood vessels develop and, as we have seen, on each side of the median plane these vessels subsequently invaginate the


roof of the *third ventricle* to form its *choroid plexuses*. The lower part of the medial wall of the cerebral hemisphere, which immediately adjoins the epithelial roof of the interventricular foramen and the anterior extremity of the diencephalon, also remains epithelial, consisting of ependyma and pia mater, while elsewhere the walls of the hemispheres are thickening to form the *pallium*. The thin part of the medial wall of the hemisphere is invaginated by vascular tissue, continuous in front with the choroid plexus of the third ventricle and constituting the choroid plexus of the *lateral ventricle*. This invagination occurs along a line which arches upwards and backwards, parallel with and initially limited to the anterior and upper boundaries of the interventricular foramen; the curved indentation of the ventricular wall, where no nervous tissue develops between ependyma and pia mater, is termed the *choroid fissure* (3.118C , 3.119A,B ) . The subsequent assumption of the complex, but exquisite, definitive form of the choroidal fissure naturally depends on related growth patterns in neighbouring structures: some are particularly relevant. These are the relatively slow growth of the interventricular foramen, the secondary 'fusion' between the lateral diencephalon and medial hemisphere walls, the encompassing of the upper brainstem by the forward growth of the temporal lobe and its pole towards the apex of the orbit and the massive expansion of two great commissures of the cerebrum—the fornix and corpus callosum. (Many of these features are detailed further below and in the Neurology section.) Nevertheless, the choroidal fissure is now clearly a caudal extension of the (much reduced) interventricular foramen, which arches above the thalamus and in this region is only a few millimetres from the median plane. Near the caudal end of the thalamus it diverges ventrolaterally, its curve reaching and continuing in the medial wall of the temporal lobe over much of its length (i.e. to the tip of the inferior horn of the lateral ventricle). The upper part of the arch is overhung by the corpus callosum and, throughout its convexity, is bordered by the fornix and its derivatives (see below). Thus, the extensive and helicoid disposition of the choroid plexus of the lateral ventricle is explained.

At first growth proceeds more actively in the floor and the adjoining part of the lateral wall of the developing hemisphere, and elevations formed by the rudimentary *corpus striatum* (3.118A ) encroach on the cavity of the lateral ventricle (Cooper 1946 ). The head of the *caudate nucleus* appears as three successive parts, medial, lateral and intermediate, which produce elevations in the floor of the lateral ventricle. Caudally these merge to form the *tail* of the caudate nucleus and the *amygdaloid complex*. From the outset the latter are close to the temporal pole of the hemisphere and, when the occipital pole grows backwards and the general enlargement of the hemisphere carries the temporal pole downwards and forwards, the tail is continued from the *floor* of the central part (*body*) of the ventricle curving into the *roof* of its temporal extension, the future *inferior horn*, and the amygdaloid complex encapsulates its tip. Anteriorly the *head* of the caudate nucleus extends forwards to the floor of the interventricular foramen, where it is separated from the developing anterior end of the thalamus by a groove; later, the head expands in the floor of the anterior horn of the lateral ventricle. The *lentiform nucleus* develops from two laminae of cells, medial and lateral, which are continuous with both the medial and lateral parts of the caudate nucleus. The *internal capsule* appears first in the medial lamina and extends laterally through the outer lamina to the cortex. It divides the laminae into two, the internal parts joining the caudate nucleus and the external parts forming the lentiform nucleus. In the latter, which consists of two main parts, the remaining medial lamina cells give rise predominantly to the (medially placed) *globus pallidus* and the lateral to the (laterally placed) *putamen*. Subsequently the putamen expands concurrently with the


intermediate part of the caudate nucleus (Hewitt 1958¹, 1961²).




As the hemisphere enlarges, the caudal part of its medial surface overlaps and hides the lateral surface of the diencephalon (thalamic part), being separated from it by a narrow cleft occupied by vascular connective tissue. At this stage (about the end of the second month) a transverse section made caudal to the interventricular foramen passes from the third ventricular cavity successively through:

- the developing thalamus
- the narrow cleft just mentioned
- the thin medial wall of the hemisphere
- the cavity of the lateral ventricle, with the corpus striatum in its floor and lateral wall (3.119A )

As the thalamus increases in extent it acquires a superior in addition to medial and lateral surfaces, and the lateral part of its superior surface fuses with the thin medial wall of the hemisphere so that, finally, this part of the thalamus is covered with the ependyma of the lateral ventricle immediately ventral to the choroid fissure (3.119B ) . As a result the corpus striatum is approximated to the thalamus and separated from it only by a deep groove which becomes obliterated by increased growth along the line of contact. The lateral aspect of the thalamus is now in continuity with the medial aspect of the corpus striatum so that a secondary union between the diencephalon and the telencephalon is effected over a wide area, providing a route for the subsequent passage of projection fibres to and from the cortex.

Throughout the brainstem, as in the spinal cord, the *migration* and differentiation of neural progenitors to form nuclei is either minimal or limited, their progeny remaining immediately extraependymal or, partially displaced towards the pial exterior, being arrested deeply embedded in the myelinated fibre 'white matter' of the region. As noted, however, the 'roof-brain' of part of the fore-, mid- and hindbrains develops following an additional, fundamental pattern which results in a superficial layer of *grey matter*. The latter consists of neuronal somata, dendrites, the terminations of incoming (afferent) axons, the stems of (or the whole of) efferent axons, geometrically and functionally apposite glial cells and vasculature. Subsequent differentiation results in a highly organized subpial surface coat of grey matter termed the *cortex* (Latin: bark, e.g. of a tree) or *pallium* (Latin: pall, mantle or cloak). Pallium is used preferentially by neuroembryologists and some comparative zoologists; however, cortex is employed much more widely and will be used here. Neither term is used in the case of the mesencephalic tectum. In the cerebral hemisphere the superficial subpial regions of its wall, both striate and supracriate (other than central areas of its medial wall, where secondary fusion with the diencephalon occurs and is encompassed by the lamina terminalis, interventricular foramen and curve of the choroid fissure), become invaded by migrating neuroblasts to form an elementary cerebral cortex. The cortical area which borders the lamina terminalis, the interventricular foramen, the convexity of the choroid fissure and continues into the diverging roots of the olfactory tract, has been simply termed the *limbic lobe* (or bordering lobe), together with its numerous subdivisions and

connections (p. 1115  et seq), the *limbic system*. Its cortical derivatives possessing, some regard, a relatively elementary structure, have been termed the *allocortex* (other cortex).

The limbic lobe is the first part of the cortex to initiate differentiation (see below) and at first it forms a continuous, almost circular strip on the medial and inferior aspects of the hemisphere. Below and in front, where the stalk of the olfactory tract is attached, it constitutes a part of the *piriform area* (*palaeocortex* or *palaeopallium*). The portion outside the curve of the choroid fissure (3.120 ) constitutes the *hippocampal formation* (archaeocortex or *archaeopallium*). In this region the neural progenitors of the developing cortex proliferate and migrate (see below), and the wall of the hemisphere thickens and produces an elevation which projects into the medial side of the ventricle. This elevation is the *hippocampus* (Humphrey 1964 , 1967 ). It appears first on the medial wall of the hemisphere in the area above and in front of the lamina terminalis (*paraterminal area*) and gradually extends backwards, curving into the region of the temporal pole where it adjoins the piriform area. The marginal zone in the neighbourhood of the hippocampus is invaded by neuroblasts forming the *dentate gyrus*. Both extend from the paraterminal area (see precommissural septum and prehippocampal rudiment) backwards above the choroid fissure and follow its curve downwards and forwards towards the temporal pole, where they continue into the piriform area. A shallow surface depression (which has been termed the *hippocampal sulcus*) grooves the medial surface of the hemisphere throughout the hippocampal formation.



The efferent fibres from the cells of the hippocampus collect along its medial edge and run forwards immediately above the choroid fissure. Anteriorly they turn ventrally and enter the lateral part of the lamina terminalis to gain the hypothalamus, where they end in and around the mamillary body and neighbouring nuclei. These efferent hippocampal fibres form the *fimbria hippocampi* and the *fornix*. For the sources of afferent fibres to the hippocampus, hippocampal commissures and multiple subdivisions of the fornix.




The terms archaeocortex and palaeocortex as the two principal divisions of the *allocortex* focus on the acceptance by earlier neuroanatomists of the phylogenetically ancient nature of these regions. The remainder of the hemispheric surface, particularly in mammals, with a relatively vast expansion in primates, is, at least at some stage in its history, a six-layered cortex, termed the *isocortex* (equal cortex) or *neocortex* (young cortex). Isocortex seems less appropriate, however, as all subregions showed fine structural differences. Furthermore, all three varieties of cortex are present simultaneously in an initial form in extant reptilia. Their relationship to piscine or amphibian ancestry remains controversial.

Development of the Commissures

The development of the commissures effects a very profound alteration on the medial wall of the hemisphere. At the time of their appearance the two hemispheres are connected to each other by the median part of the telencephalon. The roof plate of this area remains epithelial, whilst its floor becomes invaded by the decussating fibres of the optic nerves and developing hypothalamic nuclei. These two routes are thus not available for the passage of commissural

fibres passing from hemisphere to hemisphere across the median plane, and these fibres therefore pass through the anterior wall of the interventricular foramen, i.e. the lamina terminalis. The first commissures to develop are those associated with the palaeocortex and archaeocortex. Fibres of the olfactory tracts cross in the ventral or lower part of the lamina terminalis and, together with fibres from the piriform and prepiriform areas and the amygdaloid bodies, form the *anterior part* of the *anterior commissure*. In addition the two hippocampi become interconnected by transverse fibres which cross from fornix to fornix in the upper part of the lamina terminalis as the *commissure of the fornix*. Various other decussating fibre bundles (known as the *supraoptic commissures*, although they are not true commissures) develop in the lamina terminalis immediately dorsal to the optic chiasma, between it and the anterior commissure.

The commissures of the neocortex develop later and follow the pathways already established by the commissures of the limbic system. Fibres from the tentorial surface of the hemisphere join the anterior commissure and constitute its larger *posterior part*. All the other commissural fibres of the neocortex associate themselves closely with the commissure of the fornix and lie on its dorsal surface. These fibres increase enormously in number and the bundle rapidly outgrows its neighbours to form the corpus callosum (3.120 , 121 ).


The *corpus callosum* originates as a thick mass connecting the two cerebral hemispheres around and above the anterior commissure. (This site has been called the precommissural area, but this use has been rejected here because of increasing use of the adjective precommissural to denote the position of parts of the limbic lobe—prehippocampal rudiment, septal areas and nuclei, strands of the fornix in relation to the anterior commissure of the mature brain.) The upper end of this neocortical commissural area extends backwards to form the trunk of the corpus callosum. The *rostrum* of the corpus callosum develops later and separates some of the rostral end of the limbic area from the remainder of the cerebral hemisphere. Further backward growth of the trunk of the corpus callosum then results in the entrapped part of the limbic area becoming stretched out to form the bilateral septum pellucidum (Hewitt 1962 ). As the corpus callosum grows backwards it extends above the choroid fissure, carrying the commissure of the fornix on its under surface. In this way a new floor is formed for the longitudinal fissure, and additional structures come to lie above the epithelial roof of the third ventricle. In its backward growth the corpus callosum invades the area hitherto occupied by the **upper** part of the archaeocortical hippocampal formation, and the corresponding parts of the dentate gyrus (3.120 , 121 ) and hippocampus are reduced to vestiges—the *indusium griseum* and the *longitudinal striae*. However, the *postero-inferior* (temporal) archaeocortical regions of both dentate gyrus and hippocampus persist and **enlarge** because, with the forward growth of the temporal lobes, the brainstem presents a complete barrier to further extension of the corpus callosum in the median plane.

Neocortex

























The growth of the *neocortex* and its enormous expansion are associated with the initial appearance of projection fibres (corticofugal and corticopetal) during the latter part of the third month. These fibres follow the pathway provided by the apposition of the lateral aspect of the thalamus with the medial aspect of the corpus striatum, and, as they do so, they (the *internal*


capsule) divide the latter, almost completely, into a lateral part, the lentiform nucleus, and a medial part, the caudate nucleus; these two nuclei remain confluent only in their antero-inferior regions. The corticospinal tracts begin to develop in the ninth week of fetal life and have reached their caudal limits by the twenty-ninth week. The fibres destined for the cervical and upper thoracic regions and implicated in the innervation of the upper limb are in advance of those concerned with the lower limbs, which, in turn, are in advance of those concerned with the face. The appearance of reflexes in these three parts of the body shows a comparable sequence (Humphrey 1960^[4]). For further analysis of the development of the projection fibres and corpus striatum see Hewitt (1961^[4], 1962^[4]).


The preceding emphasis on corticospinal projection fibres is a reflection of the limited information available to the earlier neuroanatomists. It should be emphasized, however, that the majority of subcortical nuclear masses receive terminals from descending fibres of cortical origin. Furthermore, the foregoing are joined by thalamocortical, hypothalamocortical and other afferent ascending bundles, the whole complex constituting the internal capsule that divides the early corpus striatum. It should also be noted that the internal capsular fibres pass **lateral** to the head and body of the caudate nucleus, the anterior cornu and central part of the lateral ventricle, the rostroventral extensions and body of the fornix, the dorsal thalamus and dorsal choroidal fissure; at similar levels they pass **medial** to the lentiform nucleus. With temporal lobe formation, the capsular fibres also lie medial to the inferior cornu of the lateral ventricle which has the amygdaloid complex capping its tip, the tail of the caudate nucleus in its roof, the hippocampus, dentate gyrus and fimbria of fornix in its floor, and temporal extension of the choroidal fissure in its medial wall.

At the end of the third month the superolateral surface of the cerebral hemisphere shows a slight depression anterosuperior to the temporal pole. This corresponds to the site of the corpus striatum in the floor and lateral wall of the ventricle, and its presence is due to the more rapid growth of the adjoining cortical regions. This *lateral cerebral fossa* gradually becomes overlapped and submerged, and is converted into the *lateral cerebral sulcus*; its floor becomes the *insula* (3.122A-G ). The process, however, is not completed in its most anterior part until after birth. The presumptive neocortical areas that overlap the insula are termed the frontal, parietal and temporal *opercula*. The lentiform nucleus (lateral part of the corpus striatum) remains deep to and coextensive with the insula, the superficial zones of the latter transforming into varieties of cortex. Posteriorly the insula develops granular neocortex and an intermediate area forms agranular neocortex; finally the rostroventral area becomes similar to and continuous with the palaeocortex of the piriform area.


The growth changes in the temporal lobe which help to submerge the insula produce important changes in the olfactory and other neighbouring limbic areas. The olfactory tract, as it approaches the hemispheric floor, diverges into *lateral*, *medial* and (variable) *intermediate striae*. The medial stria is clothed with a thin archaeocortical *medial olfactory gyrus*: this curves up into further archaeocortical areas anterior to the lamina terminalis (paraterminal gyrus, prehippocampal rudiment, parolfactory gyrus, septal nuclei) and these continue into the indusium griseum. The lateral stria, clothed by the *lateral olfactory gyrus*, and, when present, the intermediate stria, terminate in the rostral parts of the *piriform area*. In brief, this includes the



olfactory trigone and tubercle, anterior perforated substance and the uncus (hook) and entorhinal area of the anterior part of the future parahippocampal gyrus. Its lateral limit is indicated by the *rhinal sulcus*. (For details of the numerous subdivisions and putative interconnections of these areas, see p. 1115  et seq and 8.229 , 8.230 , 8.231 , 8.232 , 8.233 , 8.234 , 8.235 , 8.236 , 8.237 , 8.238 , 8.239 , 8.240 , 8.241 , 8.242 , 8.243 , 8.244 , 8.245 , 8.246 , 8.247 , 8.248 , 8.249 , 8.250 .) The forward growth of the temporal pole and the general expansion of the neopallium cause the lateral olfactory gyrus to bend laterally, the summit of the convexity lying at the antero-inferior corner of the developing insula (3.122A-G ). During the fourth and fifth months much of the piriform area becomes submerged by the adjoining neopallium and in the adult only a part of it remains visible on the inferior aspect of the cerebrum.

Apart from the shallow hippocampal sulcus and the lateral cerebral fossa the surfaces of the hemisphere remain smooth and uninterrupted until early in the fourth month (3.122A-G ). The parieto-occipital sulcus also appears about that time on the **medial** aspect of the hemisphere and its appearance seems associated with the increase in the splenial fibres of the corpus callosum. Over the same period the posterior part of the *calcarine sulcus* appears as a shallow groove extending forwards from a region near the occipital pole. It is a true infolding of the cortex in the long axis of the *striate area*, producing an elevation, the *calcar avis*, on the medial wall of the posterior horn of the ventricle.

During the fifth month the *sulcus cinguli* appears on the medial aspect of the hemisphere, but not until the sixth month do sulci appear on the inferior and superolateral aspects. The *central*, *precentral* and *postcentral sulci* appear, each in two parts, upper and lower, which usually coalesce shortly afterwards although they may remain discontinuous. The *superior* and *inferior frontal*, the *intraparietal*, *occipital*, *superior* and *inferior temporal*, the *occipitotemporal*, *collateral* and *rhinal sulci* make their appearance during the same period, and by the end of the eighth month all the important sulci can be recognized (3.122A-G ).

Histogenesis of the Cortex

The histogenesis of the cortical (pallial) wall of the cerebral hemisphere has generated an impressive literature since the early 1930s. Nevertheless, because of the immense complexity, multiplicity of cell types and structural heterogeneity in different locations, descriptive and experimental analyses are less well understood and documented than those appertaining to the cerebellum, with its regular, geometrically ordered microstructure (p. 243 ). Only the briefest review of some basic principles, together with a few introductory key references, can be encompassed in this volume and the interested reader will find it apposite to constantly cross-refer to the sections devoted to mature neuronal and cortical architecture (Section 6).

The wall of the earliest cerebral hemisphere, as elsewhere in the neural tube, consists of a pseudostratified epithelium, its cells exhibiting interkinetic migration as they proliferate to form clones of, it was assumed, as yet uncommitted germinal cells. The columnar cells elongate and (following the initial nomenclature proposed by the Boulder Committee, 1970  and 3.123 )

their non-nucleated peripheral processes now constitute a *marginal zone*, whilst their nucleated, paraluminal and mitosing regions constitute the *ventricular zone*. Some of the mitotic progeny now leave the ventricular zone and migrate to occupy a *mantle (intermediate) zone*. This *proliferative phase* continues for a considerable period of fetal (and in some species postnatal) life but, as in the case of the cerebellar cortex, after a period groups of progenitor cells form, first, generations of definitive neurons and, later, glial cells which migrate to and mature in their final positions (see below for variant views). It must be appreciated, however, that these phases of proliferation, migration, differentiation and maturation are not precisely sequential for each cell variety but overlap each other in space and time.

The earliest migration of neuronal precursors from the ventricular and intermediate zones occurs radially until they approach, but do not reach, the pial surface, their somata becoming arranged as a transient *cortical plate*. Subsequently, proliferation wanes in the ventricular zone but for considerable periods persists in the immediately subjacent *subventricular zone*. From the pial surface inwards, therefore, there may now be defined the following zones: *marginal*, *cortical plate*, *subplate*, *mantle (intermediate)*, *subventricular* and *ventricular*. Briefly, whilst the foregoing **terminology** is relatively recent it has for long been accepted that the marginal zone forms the outermost layer of the cerebral cortex, the neuroblasts of the cortical plate and subplate form the neurons of the remaining cortical laminae (the complexity, of course, varying in different locations and with further additions of neurons from the deeper zones), whilst the intermediate zone gradually transforms into the white matter of the hemisphere. Meanwhile other deep progenitor cells have been producing generations of glioblasts which also migrate into the more superficial layers. As proliferation wanes and finally ceases in the ventricular and subventricular zones their remaining cells differentiate into general or specialized ependymal cells, tanycytes or subependymal glial cells. Figure 3.123A-F summarizes the modifications of the Boulder Committee's original proposals, suggested by Rakic (1982) in the light of more recent investigations.

As mentioned above, the phases of proliferation, neurogenesis and gliogenesis are by no means sequential as first envisaged but vary spatiotemporally with location and cell type. Further, the gliogenesis referred to was related to the (numerically largest) astrocyte and oligodendrocyte population of the mature tissue. However, as noted (p. 230), immunohistochemical studies of glial fibrillary acidic protein (GFAP) distribution showed a patterned array of GFAP-positive columnar cells in the earliest pseudostratified neuroepithelium of the neural tube including the walls of the rudimentary cerebral hemisphere. The positive cellular elements are interspersed with GFA-negative columnar cells, both undergoing interkinetic migration or proliferation. The GFAP-positive elements are presumptive glial cells which stretch radially across the full thickness of the wall of the telencephalon and provide contact guidance paths for the subsequent peripheral migration of neurons. Recent evidence suggests that these glial processes may be oriented in a manner that is far from strictly radial, and may instead branch extensively. The first groups of cells to migrate are destined for the deep cortical laminae, later groups passing through them to more superficial regions (see below). The subplate zone, a transient feature most prominent during midgestation, contains neurons surrounded by a dense neuropil: this subplate neuropil is the site of the most intense synaptogenesis in the cortex.

Thus, with growth, both radial and tangential, there occurs a great increase in cortical thickness and a vast increase in surface area.

Pioneering studies into cell migration in the developing mammalian *neocortex* were made by Tilney (1933^[1]), the technique available to him at this time being analysis of sections of Nissl stained tissue. Whilst it was clear that the subpial (marginal) zone formed the plexiform lamina (I), he considered that the remaining laminae stemmed from **three** quite distinct and **separate migrations** of neuroblasts up to the cortical plate. The first migration he thought differentiated into the external granular lamina (II) and the pyramidal lamina (III); the second migration forming the internal granular lamina IV; the third migration he held formed the ganglionic lamina V and the multiform lamina VI. On this view, therefore, the **outermost** layers were the **earliest** to be formed, with progressively deeper layers at successively later times.

The possibility of precisely the **reverse** sequence, progressing from *deep* to *superficial*, was first implied by the results of X-irradiation studies by Hicks et al (1959^[2]). Further irradiation studies (Berry & Eayrs 1966^[3]), and autoradiographic nuclear labelling studies (Berry & Rogers 1965^[4]; Berry 1974^[5] and 3.124^[6]) supported this contention. These seminal investigations have, in the subsequent years, been amply confirmed in the rat (3.125^[7], 126^[8]), mouse, opossum and golden hamster (Berry 1974^[5], 1982^[9]; see also comments and bibliographies in: Smart 1982^[10], 1983^[11]; Rakic & Goldman-Rakic 1982^[12]). Thus in these various mammals, apart from the pre-existing anlage of lamina I, the first laminae to be populated are VI and V, followed sequentially by laminae IV to II in 'inside–outside' fashion. Clearly, whilst the ontogenetic timings of migrations from these experimental sources are not directly appropriate to a volume on **human** anatomy, it is assumed from comparison of purely descriptive material that similar **patterns** of migration and elaboration occur in the human cortex. It should also be noted that, as yet only in the human cortex, a thin subpial lamina of densely staining cells, of unknown origin or destination, has been identified (Rabinowicz 1964^[13], 1967^[14]; Brun 1965^[15]): they are not a prominent feature of cortical histogenesis but an analogy with the external germinal layer of the cerebellum has been suggested.

No attempt will be made here further to discuss neuroblast and glioblast differentiation, migration and maturation with the establishment of intercellular contacts. However, some general hypotheses may be mentioned, involving a comparison of ontogeny and phylogeny. Firstly, all parts of the neural tube, from the presumptive spinal cord to presumptive neocortex, pass through the stage of a pseudostratified epithelium and a proliferative phase, followed by differentiation of ventricular, mantle and marginal zones. Neuroblast migration, target cell contact and maturation (or, in some locations, *degeneration* and *cell death*), whilst **still confined** to the deeper reaches of the mantle zone, are the principal events in spinal cord development. Throughout the encephalon, however, the primary difference is the **continued migration** of neuroblasts and their ultimate maturation far **beyond** the confines of the mantle zone, forming either nuclear masses, variously displaced from the ventricular and aqueductal channels, or, in the 'roof-brain' regions, reaching the subpial marginal zones forming, initially, a simple cortical plate of neuroblasts. The latter then differentiates into subzones, showing a tangential *laminar* organization, whilst in some locations there emerges a well-defined *columnar (modular)* radial

organization. Such cortical dispositions are evident in the hemispheric forebrain, tectal midbrain and cerebellar hindbrain. In the pallial walls of the mammalian cerebral hemisphere, the phylogenetically **oldest** regions and the **first** to differentiate during ontogeny are those that **border** the interventricular foramen, and its extension the choroidal fissure, the lamina terminalis and piriform lobe. There exists an increasingly complex level of organization from three to six tangential laminae, passing from the dentate gyrus and cornu ammonis through the subiculum until the general neocortex is reached. (It may be noted that many investigators find the simple progression from three to six major laminae a gross oversimplification, and numerous subdivisions have been proposed, e.g. see cornu ammonis, p. 1124 [\[1\]](#), and neocortex, p. 1141 [\[2\]](#).) The **deepest** and phylogenetically oldest *tangential laminae* are the first to be populated by migrating progenitor cells, more superficial layers being added in sequence, their neuroblasts migrating **through** the older layers; the number of superadded laminae depends upon the location with respect to the choroidal fissure. These broad patterns have been demonstrated by nuclear labelling studies, not only in the neocortex but, with modifications, also in the dentate gyrus and hippocampus (see, e.g. Angevine 1975 [\[3\]](#); Altman & Bayer 1975 [\[4\]](#); Smart 1982 [\[5\]](#)).

Mechanisms of Cortical Development

Intense interest and research effort has been concentrated on elucidating the mechanisms of development of the mammalian cerebral cortex. In the 19th century two important ideas emerged. These were, firstly, that the cortex displayed *parcellation* into a number of functional areas, and secondly, that despite differences, these various areas were organized according to a common scheme. The science of phrenology claimed to allot functions to particular bumps on the surface of the brain, an idea that was proved broadly correct by surgical ablation experiments that showed loss of particular functions. Only later in the 19th century were attempts made to correlate histological with functional observations. Staining techniques made possible the visualization of differences in the sizes, shapes and distributions of cells in various cortical areas. Comparisons of the brains of various mammals also gave rise to the idea of functional and structural homology of cortical areas in different species. This may have led to the, perhaps, simplistic view that evolution simply adds new areas to an existing schema almost without modification, giving rise to the concepts of palaeocortex, archaocortex and neocortex. Nevertheless, it is clear that a common ground plan for the cerebral cortex exists among mammals, even if in humans extensive modifications have occurred. For example, the area given over to olfactory function is reduced, and association areas are massively expanded compared with the cortices of, say, rodents. In the present century, the mechanisms of development of the areal and the laminar organization of the cortex have been investigated, in addition to studies on the cellular mechanisms of neuronal migration within the cortex, and the developing pattern of connections. Only some of the central themes of the field of cortical development will be mentioned here.

The picture that is emerging of cortical development is one in which input plays a central role, and epigenetic interactions are crucial (see reviews: O'Leary 1989 [\[6\]](#); McConnell 1992 [\[7\]](#); Shatz 1992 [\[8\]](#)). Differences may exist in the timing and control of the emergence of the laminar and the areal organization. The neocortex, which constitutes 90% of the cortical area, goes through at least a period of its development when it contains 6 layers. Palaeocortex contains the olfactory

areas, and the archaeocortex comprises the hippocampal formation. Within the 6-layered neocortex, each lamina has a distinct histology, function and connections. Layer 4 receives the major outputs from the thalamus, while layer 5 contains a high proportion of pyramidal neurons (large cells with prominent apical dendrites) with outputs to subcortical targets. Each of the layers are generated during a specific period of development in inside–outside fashion (p. 253). Rakic (1971a, b) initially demonstrated the migration of neurons along radial glial processes, and the migratory behaviour of cortical neurons was subsequently investigated in vitro (Hatten 1990). Cortical neurons or cerebellar granule cells appear equally capable of migrating on hippocampal or cerebellar Bergmann glia, indicating conservation of migration mechanisms in different brain regions. Such neurons can migrate 10 times faster than in vivo, exhibiting close apposition and forming specialized junctions with the glial process and an active advancing process that extends and retracts. Antibodies to astrotactin may disrupt neuron–glia interactions (Edmondson & Hatten 1987) and growth cones may secrete proteases that allow them to digest the extracellular matrix in their pathway (Krystosek & Seeds 1981).

Various lines of evidence point to the idea that the laminar fate of neurons is determined prior to migration. In the mutant *reeler* mouse, laminar formation is disrupted such that layers form in outside-in rather than inside-out array, yet axonal connections and neuronal properties appear normal (Caviness 1982). Laminar commitment was explored in heterochronic transplant experiments in the ferret. Since superficial cortical layers are generated later in development than deep ones, ventricular zone progenitor cells can be labelled and transplanted into host animals of a different age to investigate their laminar destination (McConnell & Kasnowski 1991). Cells from a brain in which layer 6 was being generated were labelled with 3H thymidine and transplanted into a brain in which layer 2/3 neurons were being generated. When the grafted cells had been allowed to complete their current round of division, they migrated to occupy laminae typical of their origin i.e. layer 6. When the cells were transplanted during S-phase of the cell cycle, however, so that they completed division in their new environment, the majority migrated into layer 2/3, appropriate to the host environment. Varying the time between labelling and injection showed that commitment to a particular cortical lamina occurs shortly after S-phase. This implies that cells acquire their laminar fate during certain phases of the cell cycle, depending on the environment, possibly since cells lie in the ventricular zone, adjacent to the forming white matter. Neurons of pre-existing laminae that have begun axonogenesis may provide a feedback on the forming cortical layers, providing a sort of developmental clock for histogenesis (reviewed in McConnell 1989, 1992).

In a plane perpendicular to its laminae, the cortex is divided up into a number of areas, displaying a hierarchy of organization. These include the primary areas, such as the motor cortex, the unimodal association areas concerned with the integration of information from one of the former, and multimodal association areas that integrate information from more than one modality. Besides these are the areas concerned with functions that are even less understood, such as the frontal lobes, concerned with goal-orientation responsibility and long-term planning. The primary areas are further divided up into somatotopic maps, while at the finest level, the cortex is known to consist of a series of 'columns', 50–500 µm wide. Within such a column, cells on a vertical traverse display common features of modality and electrophysiological responses to stimuli. Prominent examples of this organization are the ocular dominance columns of the visual

cortex, with cells within a column all responding to visual stimuli received from one eye.

Controversy about the development of the prominent areal organization of the cortex may be seen as hinging on the dichotomy between the idea of a cortical 'protomap', and a gradually emerging pattern that may be largely dependent on afferent input. The former idea was originated by Rakic (1988). He proposed a mosaic of small groups of progenitor cells in the ventricular zone, each of which underwent radial migration to give rise to the segregated functional columns. This appealing idea could thus explain the whole hierarchy of cortical organization, and became predominant within the field, along with its implication that the migration of progenitor cells occurs without significant tangential movement.

Several lines of evidence now suggest that the radial unit idea of cortical development must be modified. This evidence comes from descriptive studies of the movement of progenitor cells in the developing cortex, and experimental approaches that involve transplantation of presumptive regions of cortex to other locations, after which their differentiation was examined. Experiments in which progenitor cells are marked using replication-incompetent retroviruses have been used extensively to investigate cell lineages and patterns of cell migration in the cerebral cortex. Retroviral particles in suspension are injected into the fetal ventricles, and infect progenitor cells close to the ventricular surface (Sanes et al 1986; Price et al 1987). Understanding patterns of cell migration in the cortex is essential for interpretation of these experiments, which has been controversial (see Guthrie 1992). Several progenitor cells are labelled in each brain, so that definition of their clonal progeny at a later time point must be based on the coherence and separation of clones from one another. In most studies, clones of cells are radially disposed, but there are often ambiguous outliers (Luskin et al 1988). Interpretation of such cells that may have migrated tangentially away from their point of origin is subjective. They may be considered as 'single cell clones', or as sibling cells that populate separate cortical radii (Walsh & Cepko 1988). In one study, the physical displacement of supposed clonal relatives implied that migration had occurred along the processes of cortical glia that may be obliquely-oriented (Austin & Cepko 1990).

An attempt to resolve the question of clonality was made by retroviral marking experiments in which progenitor cells were marked with unique genetic tags so that their progeny could be identified by molecular techniques, irrespective of migration paths (Walsh & Cepko 1992). Despite the necessity of sophisticated statistics to show the validity of this approach, this study yielded the interesting finding that cells of the same clone dispersed as much as 1.5 mm from each other (more than 10 times the diameter of a cortical column). Furthermore, clonal relatives could populate different functional areas such as motor, visual and somatosensory cortex, as well as several units (barrels) within the somatosensory cortex. Clones arising from retrovirally-marked precursors could also cross area boundaries in the hippocampus (Grove et al 1992). The presence of significant tangential dispersion in deep ventricular or subventricular zones has also been revealed by retroviral lineage experiments in which clonal dispersion was examined in the rat at various times after labelling (Walsh & Cepko 1993). The presence of an unsuspected degree of tangential movement was also described in cortical explant cultures, by directly injecting single cells with fluorescent dye and following them with time-lapse microscopy (Fishell et al 1993). While about 82% of cells moved in a radial or near radial

direction, 13% of cells migrated rapidly in the tangential direction, often covering much larger distances than 500 μm , and making sharp right-angled turns from one pathway to the other. Interestingly, progenitor cells appeared to respect a line between the cortical and basal forebrain, raising the possibility of lineage restriction as a mechanism at least in the development of some forebrain regions. The weight of evidence now favours the idea of radial migration, with considerable tangential movement superimposed, at least of some cells. Nevertheless, some controversy exists, since in direct labelling experiments, cells could move tangentially 200 μm in 8 hours, whereas retroviral labelling experiments showed tangential movement only occurring over the course of days. Analysis of transgenic mice in which a *lacZ* transgene is inserted into one of the X chromosomes may have helped to reconcile these views (Tan & Breen 1993^[1]). In hemizygous female embryos, the transgene is inactivated in half of the cells, leading to marking of 50% of cortical progenitors. In such animals, localization of the *lacZ* gene product showed the cortex to be patterned in alternating stripes of blue or white about 100–1000 μm wide. The banding pattern thus suggests population by groups of progenitor cells that have not dispersed widely in the tangential plane. However, in each of the stripes, about one-third of cells were the inappropriate colour, suggesting that a subpopulation had migrated tangentially.

Studies of cell migration are consistent with the idea that cortical areas might not be rigidly determined. Manipulations of the developing cortex by deafferentation or manipulation of inputs have been informative as to the state of commitment of cortical areas. In two independent sets of experiments, somatosensory or auditory cortex was induced to process visual information by misrouting retinal axons to somatosensory thalamus or auditory thalamus in the neonatal ferret (Sur et al 1988^[2]). In the first case, the lateral geniculate nucleus and the visual cortex were ablated and space was created in the medial geniculate by ablating the inferior colliculus. Amazingly, cells in the somatosensory or auditory cortex were visually driven, and receptive field and response properties resembled that seen in the visual cortex. This would seem to indicate that modality of a sensory thalamic nucleus or cortical area can be specified by inputs during development.

In the somatosensory cortex of the mouse, experiments on the cytoarchitectonic units termed 'barrels' have given much information on the specification of cortical areas. These units provide a one-to-one representation of sensory vibrissae on the muzzle, forming clusters of layer 4 neurons and thalamic afferents. Barrels can be detected by histochemical staining, but are only apparent during maturation, emerging out of what appears to be the uniform cortical plate. It is now well-established that the patterning of barrels is dependent on afferent input. Injury of individual vibrissae at birth leads to absence of the corresponding barrel (Van der Loos & Wolsey 1973^[3]). Furthermore, in strains of mice with abnormal sets of vibrissae, extra barrels are present in the cortex, but only if the anomalous vibrissa receives sufficient sensory axons (Welker & Van der Loos 1986^[4]). When pieces of visual cortex are transplanted into the position of somatosensory cortex, the characteristic barrel morphology develops (Schlaggar 1994^[5]). All this points to the importance of afferents in specifying cortical areas, tempered by the idea that some area-specific properties may be determined early on. Removal of an eye in primates leads to atrophy of area 17 of the visual cortex, due to lack of 50% of lateral geniculate neurons, the major input to this region. The drastic reduction in the size of area 17 is accompanied by a shift in the position of the area 17/18 boundary, and an area of cortex normally contained within area 17 takes on the

appearance of area 18, pointing to some plasticity in the development of area-specific features. However, the laminar organization of area 17, the boundary between area 17 and 18, and the distribution of callosal projections from these areas are maintained (Dehay et al 1989^[1]).

Experiments on these questions, particularly in attempting to define the identity of transplanted regions, have suffered from a lack of area-specific markers. At present, the molecular markers available identify broader regions of the cortex, for example, the limbic system-associated membrane protein (LAMP) which is exclusive to limbic structures. Limbic regions transplanted elsewhere in the cortex maintain their expressions of this marker, perhaps arguing for early determination of this region (Barbe & Levitt 1991^[2]). Other markers are expressed differentially in the cortex relative to the adjacent forebrain areas (reviewed in Boncinelli 1994^[3]; Puelles & Rubinstein 1993^[4]). The genes *Emx-1* and *Emx-2* are expressed in neocortex but not the piriform cortex or basal ganglia, while *Dlx-1* and *Dlx-2* are expressed in the reciprocal pattern, in ventral forebrain regions including the basal ganglia (Bulfone et al 1993^[5]). *Emx-1* is expressed in the dorsal telencephalon, in a domain that is contained within that of *Emx-2*, which extends through the dorsal telencephalon and parts of the diencephalon. These expression domains are, in turn, contained within the expression domains of another gene, *Otx-1*, which is contained within that of *Otx-2* (Simeone et al 1992^[6]). Both these genes have expression domains that comprise dorsal, and most ventral domains of telencephalon, diencephalon and mesencephalon. Interestingly these 'nested' expression patterns appear during development in a sequence progressing from the most extensive domain to the least extensive, i.e. *Otx-2* is expressed first, followed by *Otx-1*, then *Emx-2*, then *Emx-1*. It is tempting to speculate, therefore, that these genes might be involved in the specification of cell fate in various brain regions.

The development of cortical projections has been investigated both in terms of laminar and area-specific connectivity. Recently, attention has focused on the idea that connections might be influenced by the existence of a transient population of subplate neurons. Studies by Marin-Padilla (1971^[7]) showed that the cortex develops within a preplate, consisting of corticopetal nerve fibres and the earliest generated neurons. This zone is then split by the arrival of cortical neurons into two zones, the subplate underneath the cortical plate, and the marginal zone at the pial surface. Subplate neurons extend axons via the internal capsule to the thalamus and superior colliculus at times before other cortical neurons have been born (McConnell et al 1989^[8]). Studies by Shatz and colleagues on the cat and the ferret have contended the subplate neurons to be a transient cell population that later dies. Thalamocortical afferents synapse with subplate neurons during their 'waiting period' prior to innervating their target in layer 4. Ablation of subplate neurons using kainic acid may cause thalamocortical afferents to fail to invade appropriate cortical areas (Ghosh & Shatz 1993^[9]). Axonal tracing studies in the rat, however, have contested the idea of a crucial role for subplate axons in thalamocortical connections. Labelling of axonal trajectories showed that subplate pathways to the internal capsule are established at about the same time as thalamocortical pathways to the thalamus, and that their trajectories in the cortex are separate (De Carlos & O'Leary 1992^[10]). More recently, the possibility that subplate axons may also play a role in the projection of cortical efferents from layer 5 and 6 has also been proposed. Examination of these axonal pathways following ablation of subplate neurons showed that in half the cases, cortical axons failed to invade their normal subcortical targets (McConnell et al 1994^[11]). In cultures of rat, visual cortical slices combined

either with cortex or with thalamus subplate neurons were not detected; however, projections to the target tissue from the appropriate lamina were formed as in vivo (Bolz et al 1990). In cortex/thalamus cultures, corticofugal cells could be labelled even in the absence of corticopetal projections. The possibility that subplate axons showed regional specificity in their connections with particular thalamic nuclei was also tested in culture experiments by confronting visual cortex with a choice of appropriate (lateral geniculate nucleus) or inappropriate thalamic tissue (Molnar & Blakemore 1991), but no preference of projection was seen, making it unlikely that a selective chemotropism governs the trajectories of subplate axons.

A crucial question is the way in which region-specific projections are generated. Layer 5 neurons in various cortical areas extend axons to different repertoires of targets. For instance, layer 5 neurons of the visual cortex project to the tectum, pons and mesencephalic nuclei, while those in the motor cortex project to mesencephalic and pontine targets, the inferior olive and dorsal column nuclei and the spinal cord. An interesting feature of these cortical projections is that they arise by collateral formation (O'Leary & Terashima 1988) rather than by projection of the primary axon, or growth cone bifurcation. In the case of the corticopontine projection, collaterals are elicited by a diffusible, chemotrophic agent (Heffner et al 1990). Retrograde labelling of neurons at various times in development has shown that rather than being generated de novo, these patterns seem to arise by pruning of collaterals from a more widespread projection. So, visual cortical neurons possess a projection to the spinal cord early in development, which is later eliminated (O'Leary & Stanfield 1985). This later emergence of specific projections could arise either by intrinsic programming of the neurons to undergo this pruning, or position-dependent factors. Heterotopic transplantation experiments have now shown that the latter is the case (O'Leary & Stanfield 1989). When pieces of visual cortex were transplanted into motor areas, and the resulting layer 5 projections labelled at later times in development, projections to the spinal cord persisted, rather than being eliminated as in normal development. Neurons in pieces of motor cortex transplanted in the place of visual cortex lost their collaterals in the same manner as the neurons of the host. Thus position plays an important role in the modelling of cortical projections, implying that the same classes of neurons exist in different tangential regions of the cortex. Presumably, the selective removal of inappropriate collaterals is governed by local factors at the site of axon termination rather than at the neuronal cell body. Nevertheless, some distinctions between neuronal classes may exist from an early stage, since cortical projection neurons are never found to possess callosal axons (Koester & O'Leary 1993). Interestingly, neurons destined to possess corticocortical axons may initially project to the opposite hemisphere, a projection that is later lost (Innocenti et al 1986). Elimination of axons to give rise to the mature distribution of callosal neurons may be affected by sensory inputs, since manipulation of thalamic inputs can lead to failure of this remodelling of callosal projections (Dehay et al 1989). Regressive events such as axon and synapse elimination and neuronal death thus play an important part in modelling the cortex. In rodents, for example, about 30% of cortical neurons die, with the number of cells in layer 4 being governed by thalamic input.

The critical role apparently played by thalamic input in organizing the regional differentiation of the cortex begs the question of how thalamic afferents are themselves organized. The possibility that each cortical area exerts a specific trophic or tropic influence on axons from the appropriate

thalamic nucleus was examined in explant slice cultures in which the laminar origin of growing axons could be visualized. When portions of lateral geniculate nucleus were cultured with a 'choice' of occipital cortex and frontal cortex (appropriate and inappropriate targets respectively) no preference in the pattern of outgrowth was observed, although axons in both targets terminated correctly, in layer 4 (Molnar & Blakemore 1991^[4]). It seems unlikely, then, that a mosaic of region-specific, possibly diffusible factors directs the thalamocortical projection. Instead, it may be that thalamocortical and corticothalamic projections reach the internal capsule simultaneously to provide a mutual guidance mechanism. Factors in the local environment between thalamus and cortex that might lead to the specificity of projections have yet to be identified.

Perinatal Brain

The state of differentiation at birth and at various postnatal stages, as seen in Golgi (metal impregnation) preparations, has been described in considerable detail elsewhere (Conel—a series of publications 1939–59^[5]). Gross nutritional deficiencies, selective neural ablation, endocrine imbalances, sensory deprivation, neurotropic viruses, vascular abnormalities and perinatal anoxia may all disturb the normal pattern of the cortex at birth. (See bibliographies in Rakic & Goldman-Rakic 1982^[6].)

At birth the volume of the brain is approximately 25% of its volume in adult life. The greater part of the increase occurs during the first year, at the end of which the volume of the brain has increased to 75% of its adult volume. The growth can be accounted for partly by increase in the size of nerve cell somata, the profusion and dimensions of their dendritic trees, axons and their collaterals and by growth of the neuroglial cells and cerebral blood vessels, but it is the acquisition of myelin sheaths by the axons which is principally responsible for it. The great sensory pathways, visual, auditory and somatic, myelinate first, the motor fibres later. During the second and subsequent years, growth proceeds much more slowly; the brain attains adult size by the seventeenth or eighteenth year. This is largely due to continued myelination of various groups of nerve fibres.

Meninges

The meningeal layers originate from paraxial mesenchyme in the trunk and caudal regions of the head, but from neural crest in regions rostral to the mesencephalon (the prechordal plate has also been suggested to make a contribution, see below). It may generally be the case that those skull bones which are formed from neural crest, e.g. the base of the skull rostral to the sella turcica, frontal, parietal and squamous temporal bones, overlie meninges which are also formed from crest cells. Certainly the work of Couly and Le Douarin supports the concept that the neural crest gives rise to the meninges over the prosencephalon.

The meninges may be divided in development into the *pachymeninx* (*dura mater*) and *leptomeninges* (*arachnoid layer*, subarachnoid space with arachnoid cells and fibres, and *pia*

mater). All meningeal layers are derived from loose mesenchyme, which surrounds the developing neural tube, termed *meninx primitiva*, or *primary meninx*. (For a detailed account of the development of the meninges in the human consult O'Rahilly & Muller 1986.)

The first indication of *pia mater*, containing the plexus of blood vessels which forms on the neural surface, is seen in the stage 11 embryo (24 days) around the caudalmost part of the medulla; this extends to the mesencephalic level by stage 12. Mesenchymal cells projecting from the rostral end of the notochord, and those in the region of the prechordal plate, extend rostrally into the mesencephalic flexure and form the earliest cells of the *tentorium cerebelli*; O'Rahilly and Muller (1986) note that at the beginning of its development the medial part of the tentorium is predominantly leptomeningeal. By stage 17 (41 days) *dura mater* can be seen in the basal areas where the future chondrocranium is also developing. The precursors of the venous sinuses lie within the pachymeninx at stage 19 (48 days), and by stage 20, cell populations in the region of the future *falx cerebri* are proliferating, although the dorsal regions of the brain are not yet covered with putative meninges.

By stage 23 (57 days) the *dura* is almost complete over the rhombencephalon and mesencephalon but is only present laterally around the prosencephalon. Subarachnoid spaces and most of the cisternae are present from this time after the *arachnoid mater* becomes separated from the primitive *dura mater* by the accumulation of cerebrospinal fluid, which now has a net movement out of the ventricular system. The medial part of the tentorium is becoming thinner. A dural component of the tentorium is seen from stage 19; the earlier developed medial portion disappears leaving a partial partition separating a subarachnoid area containing the telencephalon and diencephalon from one containing the cerebellum and rhombencephalon.

There is a very close relationship, during development, between the mesenchyme from which the cranial *dura mater* is formed and that which is chondrified and ossified, or ossified directly, to form the skull, and these layers are only clearly differentiated as the venous sinuses develop. (For an interesting study of pre- and postnatal growth of the *tentorium cerebelli*, with a mathematical analysis, see Klintworth 1967.) The relationship between the developing skull and the underlying *dura mater* continues during postnatal life while the bones of the calvaria are still growing.

The growth of the cranial vault is initiated from ossification centres within the desmocranial mesenchyme. A wave of osteodifferentiation moves radially outward from these centres stopping when adjacent bones meet, regions where sutures are induced to form. Once sutures are formed a second phase of development occurs in which growth of the cranial bones occurs at the sutural margins (Opperman et al 1993). Such growth forms most of the skull. It was proposed that the control of suture morphogenesis was sited in the *dura mater* and a variety of hypotheses have been generated to explain this process. One suggested that the *dura mater* contained fibre tracts which extended from fixed positions in the cranial base to sites of dural reflection underlying each of the cranial sutures. The tensional forces so generated would dictate the position of the sutures and locally inhibit precocious ossification. Other hypotheses support the concept of local factors in the calvaria which regulate suture morphogenesis. It has been shown clinically (and experimentally) that following removal of the entire calvaria the skull regenerates with sutures

and bones developing in anatomically correct positions, suggesting that the dura can dictate suture position in regeneration of the neonatal calvaria. Markens (1975) noted that transplantation of perinatal rat coronal suture blastema, in which the osteogenic fronts of the parietal and frontal bones had overlapped into adult host skulls, resulted in the formation of a suture which remained unossified up to 6 weeks in the host animal. Transplants of similar tissue from earlier stages did not give rise to sutures, suggesting that an osteoinhibitory message, induced in the dura mater by the interaction between the suture blastema and the advancing osteogenic front, was responsible for maintaining the transplanted sutures. This finding has been confirmed by Opperman et al (1993) who found that in transplants of sutures in which the fetal dura mater was left intact a continuous fibrous suture remained between developing vault bones, whereas in transplants in which the fetal dura mater was removed bony fusion occurred.

The presence of fetal dura is not required for the initial suture morphogenesis which appears to be controlled by mesenchymal cell proliferation and fibrous extracellular matrix synthesis induced by the overlapping of the advancing osteoinductive fronts of the calvarial bones. Opperman et al (1993) suggest that following overlap of the bone fronts a signal is transferred to the underlying dura inducing changes in localized regions beneath the sutures. Once a suture has formed, it serves as a primary site for cranial bone growth but requires constant interaction with the dura to avoid ossiferous obliteration.

Ectodermal Placodes and the Special Sense Organs

Many of the special sense organs and all of the sensory cranial nerves take origin from ectodermal placodes, regions of ectoderm containing neural progenitor cells which originate in the neural folds but remain in the surface ectoderm after neurulation (Couly 1985; see p. 222). Generally the placodal cells undergo epithelial/mesenchymal transformation after an inductive stimulus, which may be given by the proximity of the neural tube or by subjacent migration of neural crest cells, and migrate deep to the surface ectoderm to join with crest cells. Ectodermal placodes are found rostrally as the *hypophyseal*, *olfactory* and *optic placodes*, giving rise to the *adenohypophysis*, *olfactory epithelium* and *lens of the eye* respectively. More caudally the placodal cells are arranged in three main groups: ventrolateral (epibranchial), dorsolateral and intermediate. Most groups give rise, with neural crest cells, to the cranial sensory ganglia; however, the dorsolateral placode—the *otic*—gives rise to the *membranous labyrinth of the ear*, to the *acoustic ganglion*, and, with neural crest cells, to the *vestibular ganglion*. Thus the ectodermal placodes provide a significant contribution to the special sense organs in the head.

Pituitary Gland (Hypophysis Cerebri)


The hypophysis cerebri consists of the *adenohypophysis* and the *neurohypophysis* (consult p. 1883 for the varied usages of the older terms, anterior and posterior lobes, and of the more satisfactory terms adenohypophysis and neurohypophysis, and their subdivisions).






The adenohypophysis is derived, after neurulation, from placodal ectoderm of the stomodeal roof, and the neurohypophysis from the neurectoderm of the floor of the forebrain. However, chimera experimentation in chick embryos has revealed an early juxtaposition of the adenohypophyseal and neurohypophyseal populations prior to neurulation in the chick (Couly 1985^[1]) and transplantation experiments have shown similar results in amphibian embryos (Kawamura & Kikuyama 1996^[2]). At this time the neural plate has raised lateral edges, the neural folds, containing putative neural crest cells and surface ectoderm, and a midline anterior neural ridge where the neural folds converge. The most rostral portion of the neural plate, which will form the hypothalamus, is in contact rostrally with the future adenohypophysis, in the anterior neural ridge, and caudally with the neurohypophysis, in the floor of the neural plate (see 3.100^[3]). After neurulation the cells of the anterior neural ridge remain in the ectoderm and form the hypophyseal placode which is in close apposition and adherent to the overlying forebrain. Neural crest mesenchyme later moves between the prosencephalon and surface ectoderm except at the region of the placode. Before rupture of the buccopharyngeal membrane, proliferation of the periplacodal mesenchyme results in the placode forming the roof and walls of a saccular depression. This hypophyseal recess (*pouch of Rathke*; 3.127^[4], 128^[5]) is the rudiment of the adenohypophysis, lying immediately ventral to the dorsal border of the membrane, extending in front of the rostral tip of the notochord, and retaining contact with the ventral surface of the forebrain. It is constricted off by continued proliferation of the surrounding mesenchyme to form a closed vesicle, but remains for a time connected to the ectoderm of the stomodeum by a solid cord of cells, which can be traced down the posterior edge of the nasal septum. Masses of epithelial cells form mainly on each side and in the ventral wall of the vesicle, and the development of the adenohypophysis progresses by the ingrowth of a mesenchymal stroma. Differentiation of epithelial cells into stem cells and three differentiating types is said to be apparent during the early months of fetal development (Dubois 1967^[6]). It is also suggested that different types of cells arise in succession, and that they may be derived in differing proportions from different parts of the hypophyseal recess (Conklin 1968^[7]). A *cranio-pharyngeal canal*, which sometimes runs from the anterior part of the hypophyseal fossa of the sphenoid bone to the exterior of the skull, is often said to mark the original position of the hypophyseal recess (of Rathke). Traces of the stomodeal end of the recess are invariably present at the junction of the septum of the nose with the palate (see the *pharyngeal hypophysis*). Others have claimed, however, that the craniopharyngeal canal itself is a secondary formation caused by the growth of blood vessels, and is quite unconnected with the stalk of the anterior lobe (Arey 1949^[8]). Just caudal to, but in contact with, the adenohypophyseal recess a hollow diverticulum elongates towards the stomodeum from the floor of the neural plate just caudal to the hypothalamus (3.128B^[9]); this region of neural outgrowth is the neurohypophysis. It forms an *infundibular sac*, the walls of which increase in thickness until the contained cavity is obliterated except at its upper end, where it persists as the *infundibular recess* of the third ventricle. Formed in this way the neurohypophysis becomes invested by the adenohypophysis which extends dorsally on each side of it. In addition, the adenohypophysis gives off two processes from its ventral wall which grow along the infundibulum and fuse to surround it, coming into relation with the tuber cinereum and constituting the *tuberal portion* of the hypophysis. The original cavity of Rathke's pouch remains first as a cleft, and later scattered vesicles, and can be identified readily in sagittal sections through the mature gland. The dorsal wall of Rathke's pouch, which remains thin, fuses

with the adjoining part of the neurohypophysis as the *pars intermedia*.

A small endodermal diverticulum, named *Seessel's pouch*, projects towards the brain from the cranial end of the foregut, immediately caudal to the buccopharyngeal membrane. In some marsupials this pouch forms a part of the hypophysis, but in man it apparently disappears entirely.





Nose

The early development of the olfactory placodes, external nose and nasal cavities have already been considered (p. 278 .

The *olfactory nerve fibre bundles* (*fila olfactoria*) are developed from a proportion of the placodal cells which line the olfactory pits; these cells proliferate and give rise to *olfactory receptor cells*. Their central processes grow into the overlying olfactory bulb and thus form the axons of the olfactory nerves. It was claimed that the olfactory cells are from the first connected with the overlying brain by bridges of cytoplasm, within which the olfactory nerve fibres develop. More recent accounts, however, suggest that the earliest pioneer neurites are naked cytoplasmic processes which cross a mesenchyme-filled gap between the placode and the superjacent brain. Later these and subsequent generations of centrally directed neurites become enclothed in Schwann cell processes, presumably derived from the rostral neural crest (Pearson 1941 ; Van Campenhout 1956 ; Dejean et al 1958 ). Within the olfactory bulb the terminals of the olfactory axons divide repeatedly, and establish complex synaptic contacts with a number of neuroblast types in rudimentary *olfactory glomeruli* (p. 1116 ). The single dendrite extends towards the nasal cavity surface of the olfactory epithelium where, in most regions, slight expansion with surface specialization occurs (p. 1117 .

The remaining placodal cells, with probable accessions from neighbouring rostral neural crest and mixed head mesenchyme, differentiate into columnar *supporting (sustentacular) cells*, rounded *basal cells* and, by invagination, the flattened *duct-lining* and polyhedral *acinar cells* of the glands of Bowman. Later, basal infiltration by lymphocytes occurs.



Eyes

The formation of the eyes requires precisely co-ordinated development of tissues from three sources: the *neurectoderm of the forebrain* which forms the sensory retina and accessory pigmented structures, the *surface ectoderm* which forms the lens and cornea, and the intervening *neural crest mesenchyme* which contributes to the fibrous coats of the eye. Vascular tissue of the developing eye may form by local angiogenesis or vasculogenesis of angiogenetic mesenchyme (see p. 299 ). (General accounts of the development of the human eye are given by Mann 1964 ; O'Rahilly 1983 ; O'Rahilly & Muller 1987 .

Embryonic Components of the Eye


The first morphological sign of eye development is a thickening of the diencephalic neural folds at 22 days postovulation, when the embryo has 7–8 somites. This *optic primordium* extends on both sides of the neural plate, crossing the midline at the *primordium chiasmatis*. A slight transverse indentation, the *optic sulcus*, appears in the inner surface of the optic primordium on each side of the brain. During the period when the rostral neuropore closes, at about 24 days, the walls of the forebrain at the optic sulcus begin to evaginate, projecting laterally towards the surface ectoderm so that, by 25 days, the *optic vesicles* are formed. The lumen of each vesicle is continuous with that of the forebrain. Cells delaminate from the walls of the optic vesicle and, probably joined by head mesenchyme and cells derived from the mesencephalic neural crest, invest the vesicle in a sheath of mesenchyme. By 28 days, regional differentiation is apparent in each of the source tissues of the eye. The optic vesicle is visibly differentiated into its three primary parts: at the junction with the diencephalon a thick-walled region marks the future optic stalk; laterally, the tissue which will become the sensory retina forms a flat disc of thickened epithelium in close contact with the surface ectoderm; the thin-walled part of the vesicle which lies between these regions will later form the pigmented layer of the retina. The area of surface ectoderm that is closely apposed to the optic vesicle also thickens to form the *lens placode*. The mesenchymal sheath of the vesicle begins to show signs of angiogenesis. Evidence from the equivalent stage of mouse development shows that as the epithelial regions become morphologically distinct they are already differentiated at the molecular level. For example, the gene encoding the homeobox-containing transcription factor *Msx-2* is expressed in the future sensory retina and in the overlying surface ectoderm (Monaghan et al 1991^[1]) while *TRP-2/DI*, a gene encoding an early marker for melanoblasts, is expressed in the prospective pigmented retina (Steel et al 1992^[2]). Between 32 and 33 days postovulation, the lens placode and optic vesicle undergo co-ordinated morphogenesis. The lens placode invaginates, forming a pit which pinches off from the surface ectoderm to form the *lens vesicle*. (Consult Hendrix & Zwaan 1974^[3] for a detailed analysis of this process in the chick embryo.) The surface ectoderm reforms a continuous layer which will become the corneal epithelium. The lateral part of the optic vesicle also invaginates to form a cup, the inner layer of which (facing the lens vesicle) will become the *sensory retina*, and the outer layer the *pigmented retinal epithelium*. As a result of these folding movements, the two layers of the cup have what were their apical (luminal) surfaces now facing one another across the much reduced lumen, the *intraretinal space*. The pigmented layer becomes attached to the mesenchymal sheath, but the junction between the pigmented and sensory layers is less firm and is the site of pathological detachment of the retina. The two layers are continuous at the lip of the cup which, at the end of the third month, grows round the front of the lens and forms the pigmented *iris*. Between the base of the cup and the brain, the narrow part of the optic vesicle forms the *optic stalk*. The anteroventral surface of the vesicle and distal part of the stalk are also infolded, forming a wide groove—the *choroid fissure*—through which mesenchyme extends with an associated artery, the *hyaloid artery*. As growth proceeds, the fissure closes, including the artery in the distal part of the stalk. Failure of the optic fissure to close is a rare anomaly and there is always a corresponding deficiency in the choroid and iris (*congenital coloboma*).

Early Stages of Eye Development: Mechanisms

Understanding of the mechanisms by which the tissues of the human eye become determined, then shaped and patterned, depends on experiments conducted on the embryos of other vertebrate species, notably the mouse, chick and various amphibia (reviewed by Saha et al 1992 ). These experiments provide general principles, but it should not be assumed that the conclusions can be applied directly to every detail of the human case. In particular, there appear to be significant differences between the different vertebrate classes as regards the developmental plasticity and capacity for regeneration of the tissues in the eye. The development of the eye involves a series of interactions between neighbouring tissues in the head. These interactions have been studied extensively in experimental tissue combinations, but much of the older literature is unreliable because of technical difficulties in separating the tissues cleanly, the lack of unambiguous host/graft markers to determine the origins of structures developed from the combined tissues and the lack of specific molecular indicators of tissue type by which to assay the resulting development. Studies in amphibia, using improved methods, have shown that the formation of the optic vesicle is a result of interactions between the mesenchyme of the head and the adjacent neurectoderm during gastrulation and neurulation. These interactions lead to the development of the potential to form optic vesicles throughout a broad anterior domain of neurectoderm. As a result of further interactions between mesenchyme and neurectoderm, this region becomes subdivided into bilateral domains at the future sites of the eyes. The parallel process of lens determination appears to depend on an inductive influence spreading through the surface ectoderm from the rostral neural plate. During a brief period of competence, this elicits a lens-forming area of the head (consult Grainger et al 1992  for a review). As the optic vesicle forms and contacts the potential lens ectoderm reciprocal interactions occur which are necessary for the complete development of both tissues.

Differentiation of the Functional Components of the Eye

The developments described above bring the embryonic components of the eye into the spatial relationships necessary for the passage, focusing, and sensing of light. The next phase of development involves further patterning and cell-type differentiation in order to develop the specialized structures of the adult organ.

The *optic cup* becomes patterned, from the base to the rim, into regions with distinct functions (3.129A-C ). The external stratum remains a rather thin layer of cells which, around 36 days, begin to acquire pigmented melanosomes and form the *pigmented epithelium of the retina*. In a parallel process which was already begun before invagination, the cells of the inner layer of the cup proliferate to form a thick epithelium. The inner layer forms neural tissue over the base and sides of the cup and non-neural tissue around the lip. The non-neural epithelium is further differentiated into the components of the prospective iris at the rim and the ciliary body, a little further back adjacent to the neural area. The development of this pattern is reflected in regional differences in the expression of various genes which encode transcriptional regulators and are therefore likely to play key roles in controlling and coordinating development. Each of these genes is expressed prior to overt cell-type differentiation. For example, in the mouse embryo, the

genes *Msx-2* and *Dlx-1* are expressed in the prospective neural retina and *Msx-1* in the ciliary epithelium. In mouse and human, *Pax-6* is expressed in the prospective ciliary and iris regions of the optic cup. Individuals heterozygous for mutations in *Pax-6* lack an iris, suggesting a causal role for this gene in the development of the iris. Each of these genes, in addition to being expressed in the eye, is also active at a variety of other specific sites in the embryo. This may, in part, account for the co-involvement of the eye and other organs in syndromes which result from single genetic lesions.

The Developing Neural Retina

This comprises an outer *nuclear zone* and an inner *marginal zone*, devoid of nuclei. Around 36 days the cells of the nuclear zone invade the marginal zone, and by 44 days the nervous stratum of the retina consists of inner and outer *neuroblastic layers*. The inner neuroblastic layer gives rise to the ganglion cells, the amacrine cells and the somata of the 'fibrous' sustentacular cells (of Muller); the outer neuroblastic layer is the source of the horizontal and rod-and-cone bipolar neurons and probably the rod-and-cone cells, which first appear in the central part of the retina. By the eighth month all the named layers of the retina can be identified. However, the retinal photoreceptor cells continue to form after birth, generating an array of increasing resolution and sensitivity (Banks & Bennett 1988^[1]). (For bibliographies on retinal development, including ultrastructural studies, consult Spira & Hollenberg 1973^[2]; Fisher & Linberg 1975^[3].)

Experiments on chick embryos indicate that the divergent differentiation of the pigmented and sensory layers of the retina depends on interactions mediated by diffusible molecules. For example, soluble factors from the retina elicit the polarized distribution of plasma membrane proteins and the formation of tight junctions in the pigmented epithelium (Rizzolo & Li 1993^[4]). Neural retinal differentiation appears to be mediated by fibroblast growth factors (Pittack et al 1991^[5]; Guillemot & Cepko 1992^[6]). Even after specific differentiation is under way in the pigmented epithelium, however, this tissue retains the potential to become neural retina and will do so if the embryonic retina is wounded.

The development of specific types of cell in the retina depends on cell interactions, rather than cell lineage. In the mouse, for example, a single retinal precursor cell can give rise to at least three different types of neuron or two types of neuron and a glial cell (Fields-Berry et al 1992^[7]) and, in frog embryos, the different types of cells in the retina can be generated without cell division (Harris & Hartenstein 1991^[8]). These interactions are mediated, at least in part, by diffusible factors which are likely to act over short range, coordinating the development of neighbouring cells (Wilkinson et al 1989a^[9]; Watanabe & Raff 1992^[10]; Mudhar et al 1993^[11]). Fundamental aspects of the mechanisms by which cell signalling determines the pattern of neural cell differentiation are also becoming evident from studies which indicate the expression, in the mammalian retina, of genes that are known to be involved in spatial determination in invertebrates; examples are the mouse genes related to the *Drosophila* gene *Notch* (Reaume et al 1992^[12]) and *Achaete-Scute* (Guillemot & Joyner 1993^[13]).

Optic Nerve

The optic nerve develops from the optic stalk. The centre of the optic cup, where the optic fissure is deepest, will later form the *optic disc*. Here the neural retina is continuous with the corresponding invaginated cell layer of the optic stalk and, as a result, the developing nerve fibres of the ganglion cells pass directly into the wall of the stalk, converting it into the optic nerve. The fibres of the optic nerve begin to acquire their myelin sheaths shortly before birth, but the process is not completed until some time later. The *optic chiasma* is formed by the meeting and partial decussation of the fibres of the two optic nerves in the ventral part of the lamina terminalis at the junction of the telencephalon with the diencephalon in the floor of the third ventricle. Beyond the chiasma, the fibres are continued backwards as the optic tracts, principally to the lateral geniculate bodies and to the superior tectum.



Ciliary Body

The ciliary body is a compound structure; its epithelial components comprise the region of the inner layer of the retina between the iris and the neural retina together with the adjacent outer layer of pigmented epithelium. The cells in this region differentiate in close association with the surrounding mesenchyme to form highly vascularized folds that secrete fluid into the globe of the eye (reviewed in Bard 1990^[1]). The inner surface of the ciliary body also forms the site of attachment of the lens (see below), while the outer layer is associated with smooth muscle derived from mesenchymal cells in the choroid located between the anterior scleral condensation and the pigmented ciliary epithelium. This *ciliary muscle* functions to focus the lens.

Iris

The iris functions to regulate the aperture of the eye. It develops from the tip of the optic cup where the two layers remain thin and are associated with vascularized, muscular connective tissue. The muscles of the *sphincter* and *dilator pupillae* are unusual in being of neuroectodermal origin, developed from the cells of the pupillary part of the optic cup. The mature colour of the iris develops after birth and depends on the relative contributions of the pigmented epithelium on the posterior surface of the iris and the chromatophore cells in the mesenchymal stroma of the iris. If only epithelial pigment is present, the eye appears blue; if there is an additional contribution from the chromatophores, the eye appears brown.

Lens

This is developed from the lens vesicle (3.129A ). The vesicle is initially a ball of actively proliferating epithelium typically enclosing a clump of disintegrating cells. By 37 days, however, there is a clear difference between the thin anterior (i.e. outward facing) epithelium and the thickened posterior epithelium. Cells of the posterior wall lengthen greatly and fill the vesicle (3.129B,C ) so that, by about 44 days the original cavity is reduced to a slit. The posterior cells become filled with a very high concentration of proteins (crystallins), which render them transparent. The cells themselves become densely packed within the lens as *primary lens fibres*. Cells at the equatorial region of the lens also elongate and add additional *secondary lens fibres* to the body of the lens in a process which continues into adult life. Characteristic ultrastructural

changes during lens cell development have been described by Wulle and Lerche 1967^[4]. This process is sustained by continued proliferation of cells in the anterior epithelium. In the chick and mammal, the polarity and growth of the lens appear to depend on the differential distribution of soluble factors which promote either cell division or lens fibre differentiation and are present in the anterior chamber and vitreous humour respectively (Hyatt & Beebe 1993^[5]; Schultz et al 1993^[6]).


The developing lens is surrounded by a vascular mesenchymal condensation, the *vascular capsule*, the anterior part of which is named the *pupillary membrane*. The blood vessels supplying the posterior part of this capsule are derived from the *hyaloid artery*, those for the anterior part from the *anterior ciliary arteries*. By the sixth month all the vessels of the capsule are atrophied except the hyaloid artery, which becomes occluded during the eighth month of intrauterine life. The atrophy of both the hyaloid vasculature and the pupillary membrane appears to be an active process of programmed tissue remodelling dependent on macrophages (Lang & Bishop 1993^[7]). Prior to this, during the fourth month, the hyaloid artery gives off retinal branches and its proximal part persists in the adult as the *central artery of the retina*, together with its accompanying central vein (for details, consult Penfold et al 1990^[8]). The *hyaloid canal*, which carries the vessels through the vitreous, persists after the vessels have become occluded. In the newly born child it extends more or less horizontally from the optic disc to the posterior aspect of the lens but when the adult eye is examined with a slit-lamp it can be seen to follow a wavy, curvy course, sagging downwards as it passes forwards to the lens (Mann 1927^[9]). With the loss of its blood vessels the vascular capsule disappears and the lens becomes dependent for its nutrition on diffusion via the aqueous and vitreous humours. The lens remains enclosed, however, in the *lens capsule* which is a thickened basal lamina derived from the lens epithelium. Sometimes the pupillary membrane persists at birth, giving rise to *congenital atresia of the pupil*.

Vitreous Body


The vitreous body develops between the lens and the optic cup as a transparent, avascular gel of extracellular substance; the precise derivation of the vitreous is controversial. The lens rudiment and the optic vesicle are at first in contact, but after closure of the lens vesicle and formation of the optic cup they draw apart, remaining connected by a network of delicate cytoplasmic processes. This network, derived partly from cells of the lens and partly from those of the retina, is the *primitive vitreous body*. At first these cytoplasmic processes are connected to the whole of the neuroretinal area of the cup, but later they are limited to the ciliary region where, by a process of condensation, they form the basis of the suspensory ligaments of the *ciliary zonule*. The vascular mesenchyme which enters the cup through the choroidal fissure and around the equator of the lens associates locally with this reticular tissue and thus contributes to the formation of the vitreous body.


Aqueous Chamber

This chamber of the eye develops in the space between the surface ectoderm and the lens which is invaded by mesenchymal cells of neural crest origin. The aqueous chamber initially appears as

a cleft in this mesenchymal tissue. The mesenchyme superficial to the cleft forms the *substantia propria* of the cornea, which deep to the cleft forms the mesenchymal stroma of the iris and the pupillary membrane. Tangentially, this early cleft extends as far as the *iridocorneal angle* where communications are established with the sinus venosus sclerae. When the pupillary membrane disappears the cavity continues to form between the iris and the lens capsule as far as the zonular suspensory fibres. Thus the aqueous chamber is now divided by the iris into *anterior* and *posterior chambers*, communicating through the pupil. Their walls furnish the sites of production, and channels of circulation and reabsorption of the aqueous humour (p. 1349 )

Cornea




The cornea is induced in front of the anterior chamber by the lens and optic cup. The corneal epithelium is formed from the surface ectoderm and the epithelium of the anterior chamber from mesenchyme (O'Rahilly & Meyer 1959 )

Between the two is established a regular array of collagen fibres which serve to reduce scattering of light entering the eye (for a review of the process in the chick see Bard 1990 )

Choroid and Sclera




These differentiate as inner, vascular, and outer, fibrous, layers from the mesenchyme surrounding the optic cup. The blood vessels of the choroid develop from the fifteenth week and include the vasculature of the ciliary body. The choroid is continuous with the internal sheath of the optic nerve which is a part of the pia-arachnoid of the brain. Outside this, the sclera is continuous with the outer sheath of the optic nerve, and thus with the dura mater of the brain. The continuity of the subarachnoid space with the sheath of the optic nerve makes the optic disc and the venous return from the retina sensitive to pathological changes in the pressure of the cerebrospinal fluid.

Eyelids






The eyelids are formed as small cutaneous folds (3.129C ). About the middle of the third month their edges come together and unite over the cornea, enclosing the *conjunctival sac*. They are usually said to remain united until about the end of the sixth month. When the eyelids open, the conjunctiva lines their inner surfaces and the white (scleral) region of the eye. For a detailed account consult Sevel (1988 ) and for an account of eyelid development in the mouse see Findlater et al (1993 )

Lacrimal Apparatus

The epithelium of the alveoli and ducts of the *lacrimal gland* arise as a series of tubular buds from the ectoderm of the superior conjunctival fornix; these buds are arranged in two groups, one forming the gland proper and the other its palpebral process. The *lacrimal sac* and *nasolacrimal duct* are considered to be derived from the ectoderm in the nasomaxillary groove

between the lateral nasal prominence and the maxillary prominence (p. 237). This thickens to form a solid cord of cells which sinks into the mesenchyme; during the third month the central cells of the cord break down and a lumen is acquired. In this way the nasolacrimal duct is established. The *lacrimal canaliculi* arise as buds from the upper part of the cord cells and, secondarily, establish openings (*punctua lacrimalia*) on the margins of the lids; the inferior canaliculus cuts off a small part of the lower eyelid to form the *lacrimal caruncle* and *plica semilunaris*. The epithelium of the cornea and conjunctiva is of ectodermal origin, as are also the eyelashes and the lining cells of the tarsal, ciliary and other glands which open on the margins of the eyelids. For general accounts of ocular developmental abnormalities consult Dejean et al (1958); Mann 1964.

Ears

The rudiments of the *internal ears* appear shortly after those of the eyes as two patches of thickened, surface epithelium, *otic placodes*, lateral to the hindbrain (see p. 148). Each placode invaginates as an *otic pit* while at the same time giving cells to the *stato-acoustic* (*vestibulocochlear*) *ganglion* (3.96A). Studies have indicated that the vestibulocochlear ganglion is formed entirely by placodal cells and also that placodal cells populate the acoustic ganglion entirely and the vestibular ganglion *with* a small contribution from neural crest cells (D'Amico-Martel & Noden 1983). The mouth of the pit then closes forming an initially piriform *otocyst* (*auditory* or *otic vesicle*) from which the epithelial lining of the *membranous labyrinth* is derived (3.130A-F). A vertical infolding of its wall progressively marks off a tubular diverticulum on the medial side, which differentiates into the *ductus* and *saccus endolymphaticus*, and they communicate via the ductus with the remainder of the vesicle—the *utrículosaccular chamber*—which is placed laterally. From the dorsal part of this chamber three compressed diverticula appear as disc-like evaginations; the central parts of the walls of the discs coalesce and disappear while the peripheral portions of the discs persist as *semicircular ducts*; the anterior duct is completed first, and the lateral last. From the ventral part of the utrículosaccular chamber arises a medially directed evagination which progressively coils as the *cochlear duct*; its proximal extremity constricts as the *ductus reuniens*. The central part of the chamber now represents the membranous vestibule, divided into a smaller ventral *sacculle* and a larger *utricle* mainly by horizontal infolding which extends from the lateral wall towards the opening of the ductus endolymphaticus, leaving only a narrow *utrículosaccular duct* between its divisions. This duct becomes acutely bent on itself, its apex being continuous with the ductus endolymphaticus. During this period the membranous labyrinth undergoes a rotation so that the long axis, originally vertical, becomes more or less horizontal (Bast & Anson 1949). Subsequently, otocyst derived cells, having contributed placodal cells to the vestibulocochlear ganglion, differentiate into the specialized paraneuronal hair cells of the utricle, sacculle, ampullae of the semicircular ducts, and organ of Corti; they also differentiate into various specialized sustentacular cells and the unique epithelia of the stria vascularis and endolymphatic sac. The remainder form the general epithelial lining of the rest of the membranous labyrinth.

The mesenchyme surrounding the various parts of the epithelial labyrinth is converted into a *cartilaginous otic capsule*, and this is finally ossified to form most of the *bony labyrinth* of the

internal ear. (Exceptions are the modiolus and osseous spiral lamina—see below.) For a time the cartilaginous capsule is incomplete and the cochlear, vestibular and facial ganglia are situated in the gap between its canalicular and cochlear parts. These ganglia are soon covered by an outgrowth of cartilage and at the same time the facial nerve is covered in by a growth of cartilage from the cochlear to the canalicular part of the capsule. In the embryonic connective tissue between the cartilaginous capsule and the epithelial wall of the labyrinth the perilymphatic spaces are developed. The rudiment of the *periotic cistern* or vestibular perilymphatic space can be seen in an embryo of from 30 to 40 mm in length in the reticulum between the sacculle and the fenestra vestibuli. The scala tympani is next developed and begins opposite the fenestra cochleae; the scala vestibuli is the last to appear (Streeter 1917^[1]). The two scalae gradually extend along each side of the ductus cochlearis, and when they reach the tip of the ductus a communication, the *helicotrema*, is developed between them. The modiolus and the osseous spiral lamina of the cochlea are not preformed in cartilage but ossified directly from connective tissue.

Auditory Tube and Tympanic Cavity

These are developed from a hollow, termed the *tubotympanic recess* (Frazer 1914^[2]), between the first and third pharyngeal arches, the floor of the recess consisting of the second arch and its limiting pouches. By the forward growth of the third arch the inner part of the recess is narrowed to form the tubal region, and the inner part of the second arch is excluded from this portion of the floor. The more lateral part of the recess subsequently develops into the *tympanic cavity* and the floor of this part forms the lateral wall of the tympanic cavity up to about the level of the chorda tympani nerve. From this it will be seen that the lateral wall of the tympanic cavity contains first and second arch elements, the first arch being limited to the part in front of the anterior process of the malleus. The second arch forms the outer wall behind this and turns on to the back wall to take in the tympanohyal region. Some observations, however, indicate that the tympanic cavity is derived wholly from the first pouch (Kanagasuntheram 1967^[3]). The tubotympanic recess is at first inferolateral to the cartilaginous otic capsule, but as the latter enlarges the relations become altered and the tympanic cavity becomes anterolateral. A cartilaginous process grows from the lateral part of the capsule to form the tegmen tympani and it curves caudally to form the lateral wall of the auditory tube. In this way, subsequent to ossification, the tympanic cavity and the proximal part of the auditory tube become included in the petrous region of the temporal bone. During the sixth or seventh month the mastoid antrum appears as a dorsal expansion of the tympanic cavity. Much of the cavity's basic development thus occurs during *fetal* life (Bok 1966^[4]). A study of the posterior part of the tympanic cavity and in particular of the sinus tympani, a recess between the promontory and pyramid (p. 1373^[5]), emphasizes the late fetal development of this region (Bollobas & Hajdu 1975^[6]).


The opinion long held as to the development of the auditory ossicles was that the *malleus* derived from the dorsal end of the ventral mandibular (Meckel's) cartilage and the *incus* from the dorsal cartilage, probably corresponding to the quadrate bone of birds and reptiles. The *stapes* stems mainly from the dorsal end of the cartilage of the second (hyoid) arch, first as a ring (*annulus stapedis*) encircling the small stapedial artery (p. 314^[7]). The primordium of the stapedius muscle appears close to the artery and facial nerve at the end of the second month, and

at almost the same time the tensor tympani begins to appear near the extremity of the tubotympanic recess (Candiollo & Levi 1969^[1]). Detailed analysis of early embryos concerning the *mesenchymal* origins of the blastemal ossicles, however, differs from the foregoing. Each ossicle has at least **two** distinct sources (see p. 277^[2] and Hanson et al 1962^[3]).


At first the ossicles are embedded in the mesenchymal roof of the tympanic cavity and their extraneous origin is indicated in the adult by the covering which they receive from its mucous lining.

External Ear

The external acoustic meatus is developed from the dorsal end of the hyomandibular or first pharyngeal groove. Close to its dorsal extremity this groove extends inwards as a funnel-shaped *primary meatus* from which the cartilaginous part and a small area of the roof of the osseous meatus are developed. From this funnel-shaped tube a solid epidermal plug extends inwards along the floor of the tubotympanic recess; by the breaking down of the central cells of this plug the inner part of the meatus (*secondary meatus*) is produced, while its deepest ectodermal cells form the epidermal stratum of the *tympanic membrane*. The fibrous stratum of the membrane is formed from the mesenchyme between the meatal plate and the endodermal floor of the tubotympanic recess.

The development of the *auricle* is initiated by the appearance of six hillocks which form round the margins of the dorsal portion of the hyomandibular groove. Of the six, three are on the caudal edge of the mandibular arch and three on the cranial edge of the hyoid arch (3.142F^[4] ). These hillocks appear at the 4 mm stage but they tend to become obscured as development proceeds and of those on the mandibular arch only the most ventral, which subsequently forms the *tragus*, can be identified throughout. The remainder of the auricle follows proliferation of the mesenchyme of the hyoid arch (Streeter 1922^[5]), which extends forwards round the dorsal end of the remains of the hyomandibular groove, forming a keel-like elevation—the forerunner of the *helix*. The contribution made by the mandibular arch to the auricle is greatest at the end of the second month; as growth continues, it is relatively reduced; eventually the area of skin supplied by the mandibular nerve extends little above the tragus. The lobule is the last part of the auricle to develop.

The rudiment of the eighth nerve appears in the fourth week as the *vestibulocochlear ganglion*, which lies between the otocyst and the wall of the hindbrain. At first it is fused with the ganglion of the facial nerve (*acousticofacial ganglion*) but later the two separate. The cells of the vestibulocochlear ganglion are mainly derived from the placodal ectoderm (see above); the ganglion divides into *vestibular* and *cochlear parts*, each associated with the corresponding division of the eighth nerve. The cells of these ganglia remain bipolar throughout life, each sending a proximal fibre into the brainstem, and a peripheral fibre to the internal ear. These neurons are also unusual in that many of their *somata* become enveloped in thin *myelin sheaths*.

The ganglionic fibres just described provide, of course, the afferent, sensory innervation of the labyrinthine hair cells. The latter soon become associated with the *outgrowing* axons from cells of the superior olivary complexes of the pons which provide an efferent innervation, the *olivocochlear bundle* (p. 1394 ). Development details are, however, lacking in mankind.

Musculoskeletal System

The development of the musculoskeletal system is complex, requiring the coordinated integration of mesenchymal derivatives from different parts of the embryo and a variety of epithelial/mesenchymal interaction. Three distinct subpopulations of mesenchyme produce the majority of the system.

- *Paraxial mesenchyme* gives rise to the striated muscle throughout the head, trunk and limbs, virtually exclusively via the somites or preoccipital somitomeres (although axial mesenchyme from the prechordal plate produces the extrinsic eye muscles).
- *Somatopleuric mesenchyme* and a discrete portion of each somite, in the main, give rise to the skeletal elements, ligaments, tendons, fasciae, muscular and dermal connective tissue throughout the trunk and limbs. The former also patterns the development of the nerves, muscles and blood vessels in these locations.
- *Neural crest mesenchyme* produces the skeletal elements of the viscerocranium and much of the neurocranium, the ligaments, tendons, fasciae and muscular connective tissue throughout the head, including the meninges and dermal connective tissues. The neural crest also patterns the development of the nerves, muscles and blood vessels in these locations.

Germinal epithelia, which provide the populations of mesenchyme cells for these fates, are generated locally in the somites, each of which provides a discrete germinal epithelial plate for the production of myoblasts, and more extensively in the proliferating somatopleuric mesothelium. Neural crest cells proliferate as they migrate and also in situ. In all cases epithelial tissue close to the mesenchyme, often specifically ectoderm, contributes to the developmental processes by initiating some differentiation pathways and preventing others.

Because of the diversity of cell populations which contribute to the musculoskeletal system, its development will be considered in the following order:

(1) Development of the Axial Structures

—development of the musculoskeletal tissues of the trunk, i.e. the vertebral column and associated muscles. These structures are formed by the paraxial mesenchyme which surrounds the neural tube and notochord, and laterally by somatopleuric mesenchyme.

—development of the musculoskeletal tissues of the head, i.e. the skull and associated muscles. These structures are formed by several mesenchymal populations, i.e. a specialized portion of the axial musculoskeletal tissue, a mesenchymal population from the prechordal plate and a significant mesenchymal population from the ectodermal neural crest.

(2) Development of the Appendicular Structures

The musculoskeletal tissues of the limbs are formed from both somatopleuric mesenchyme and paraxial mesenchyme.

Our understanding of the general development of the connective and muscular tissues in the skeletal system has improved significantly with the advances in molecular biology and it is possible to see common developmental pathways which are followed by all myoblasts, chondroblasts or fibroblasts, etc. regardless of their site of origin. A brief account of some of these basic mechanisms may assist the interpretation of more specific events.

General Development of Connective Tissue Cells

The most fundamental facet of connective tissue differentiation is the production of mesenchymal condensations which, according to Atchley and Hall (1991^[1]), are the basic units from which morphology is constructed during development. Five developmental criteria identify a condensation:

- the number of stem cells
- the time of condensation initiation
- the mitotically active fraction
- the rate of cell division
- the rate of cell death.

These criteria may vary individually or in concert producing variability in developmental processes. A condensation is the first cellular product of epithelial/mesenchymal tissue interactions. (For a general account of epithelial/mesenchymal interactions, see p. 110^[2].) The formation of a mesenchymal condensation is associated with formation of gap junctions that allow intercellular communication followed by production of extracellular matrix molecules, if sufficient cells are associated within a condensation (Hall & Miyake 1992^[3]). The type and quantity of the matrix can induce and maintain production of further matrix molecules by competent cells. Particular matrix molecules are associated with specific developmental lineages and can be used to distinguish different cell fates, for example an osteogenic fate from a chondrogenic.

It is not yet clear how cells are committed to a connective tissue lineage; however, it has been

shown that single mesenchyme cells will differentiate into chondroblasts if they are maintained in a rounded configuration. Connective tissue develops from mesenchyme of different origins, for example from somatopleuric mesenchyme, cephalic neural crest cells and parts of the somite (splanchnopleuric mesenchyme also in association with the viscera). The formation of cartilage has been extensively studied; however, less is known about the development of the widespread range of connective tissue or the origin of the osteoblastic lineage. Chondrogenesis is generally initiated from mesenchyme in response to an extracellular matrix mediated interaction, either via a basal lamina as in the sclerotomes (see below), or via an ectodermal mesenchymal interaction as in the limbs and facial processes (see below). Sclerotomal cells are already determined to a chondrogenic lineage, perhaps even before somite formation; interaction with the basal laminae of the notochord and neural tube enhances the differentiation process. The mesenchyme of the limb requires both the presence of an ectodermal sleeve early in development and then subsequent interaction with extracellular matrix products for both chondrogenic and fibrocyte differentiation. A high cell density in the core of the limb is required for chondrogenic differentiation whilst an antichondrogenic zone immediately beneath the ectoderm seems to prevent the differentiation of cartilage within the dermis and myogenic zone. Limb buds cultured in the coelomic cavity usually chondrify in their peripheral zones where the ectoderm is lacking or replaced by another kind of epithelium (Brand et al 1985). The ectoderm is believed to produce matrix molecules which encourage cell flattening and fibrogenic differentiation (Christ et al 1986). Expression of type II collagen in mesenchyme cells is often a sign of terminal differentiation along a chondrogenic lineage. The ultimate fate of such cells is production of type X collagen; when this occurs the cells hypertrophy and will ultimately die. Hypertrophied cells can start the mineralization process within the expanded cartilage lacunae. Regions of persistent cartilage, (e.g. trachea, pinna, etc.) do not permit the final differentiation fate of the cell line.

The factors promoting osteoblast development are not clear. Osteogenesis coincides with the vascularization of either a cartilage model, as in *endochondral ossification*, or of a mesenchymal condensation directly, as in *intramembranous ossification*. Ossification occurs at a much slower rate than chondrogenesis (for a fuller description of these processes see p. 471). Chondroclasts and osteoclasts have been identified in older developing limbs remodelling developing bone and cartilage; they may represent the same cell line in different locations (Jacob et al 1986). Adipocytes are also related to chondroblasts and osteoblasts and fibroblasts.

General Development of Skeletal Muscle

A myogenic lineage, noted by the expression of myogenic determination factors, can be demonstrated transitorily in some cells shortly after their ingress through the primitive streak. The skeletal muscle found throughout the body is derived from the paraxial mesenchyme which segments to form the somites (see also development of the extrinsic ocular muscles).

Cells committed to a myogenic lineage will undergo a series of proliferative mitotic divisions prior to passage through a *terminal division* resulting in their restriction as *postmitotic myoblasts*. Postmitotic myoblasts can begin to transcribe the mRNAs for the major contractile proteins *actin* and *myosin* as well as a number of regulatory proteins of muscle contraction. Finally postmitotic myoblasts will assume a characteristic spindle shape and begin to fuse with one another, creating


a tube-like *syncytium*, the *myotube*. (Interestingly myoblasts from different vertebrates will fuse to form hybrid myotubes.) Subsequent to fusion, *myofibrils* assemble in the periphery of the myotube. The early myofibrils develop the cross-striated organization first at the Z line, an α -actinin rich structure that anchors the actin filaments to form the I-Z-I complexes, and later in the A band region, occupied by the myosin filaments. Sarcomere formation proceeds from the periphery towards the centre of the myotubes. When this process is complete, the nuclei migrate from the centre to the periphery, and the syncytium is now called a *myofibre*. Myofibrils align laterally with one another, the sarcoplasmic reticulum and T-tubules become arranged in transverse orientation, and the myofibre continues to grow by splitting of myofibrils as well as by addition of new myofibrils.




During development at least three populations of myoblast are formed. Embryonic myoblasts give rise to primary myotubes and muscle fibres, and thus embryonic muscle. Subsequently smaller, secondary myotubes and muscle fibres arise from late myoblasts. Finally satellite cells which are also contained within the basal lamina differentiate. These latter cells may divide in postnatal life to provide new myoblasts to fuse with the muscle fibres ensuring growth of the muscle. For more information on subsets of muscle fibres consult Section 7.

The development of the central nervous system is crucial for normal formation of the *fetal* myoblast lineage. Formation of secondary fibres appears to be nerve dependent; the number of secondary fibres is reduced by denervation. It is suggested that secondary myotubes are initiated only at sites of innervation of primary myotubes.

Axial Skeleton and Muscles

Somitogenesis

Cells destined to become paraxial mesenchyme ingress through the lateral aspect of the primitive node and rostral primitive streak (3.42 ); the mesenchyme cells thus formed retain contact with both the epiblast and hypoblast basal laminae as they migrate to their paraxial position and this persists for some time after reaching their destination.

After the onset of neurulation the paraxial mesenchyme caudal to the otic vesicle undergoes segmentation (3.131 ) in a craniocaudal progression forming discrete clusters of mesenchyme cells; this stage is termed *compaction*. In each tight cluster of paraxial mesenchyme the cells re-establish juxtaluminal junctions and organize themselves into an *epithelial somite*. The cells of the epithelial somite are polarized with respect to a central lumen which contains some mesenchymal core cells. The Golgi apparatus and mitotic figures are located in the apical region of the cells, as are actin and α -actinin; cilia develop on the free surface. The cells are joined by tight junctions and the basal surface rests on a basal lamina containing collagen, laminin, fibronectin and cytotactin (Keynes & Stern 1988 ). Processes from the somite cells pass through this basal lamina to contact the basal laminae of the neural tube and notochord (Hay 1968 ). A variety of cell adhesion molecules have been demonstrated in epithelial somites. It is worth

noting that a single somite can be described as having six faces, like a cube; it is now apparent that each facet has a slightly different fate. Further, the position along the embryo may alter the developmental fate of parts of the somite.




The epithelial somite undergoes rapid development in the following manner: the cells of the ventromedial wall seem to be pulled towards the notochord, and despite extensive juxtaluminal junctions the cells break apart. The newly formed mesenchymal cells, collectively termed the *sclerotome*, migrate medially towards the notochord; they will give rise to the bones, joints and ligaments of the vertebral column (see below). The remaining cells of the somite are now termed the *epithelial plate of the somite* or the *dermomyotome*. This epithelium produces the cell lines which will give rise to (nearly) all the striated muscles of the body. Three separate myogenic lines can be seen. Firstly, cells produced along the *craniomedial edge of the epithelial plate* elongate from the cranial to the caudal edge on the underside of the basal lamina of the plate; they are collectively termed the *myotome*. (The latter term was previously used to describe *all* the muscle forming cells of the somite; now it is usually restricted to cells deriving from the craniomedial edge.) They will give rise to the skeletal muscle dorsal to the vertebrae, the epaxial musculature (see below). Secondly, after initiation of the myotome, cells produced from the *ventrolateral edge of the epithelial plate*, opposite the limb bud, migrate into the developing limb to give rise to its skeletal muscle (see below). Lastly, the *remaining epithelial plate* (and underlying myotome cells) *grows into the flank region of the body*. The epithelial plate is still proliferating at the beginning of this stage. Later the epithelial plate cells revert to mesenchyme and processes from contiguous somites fuse to form a unified premuscular mass which gives rise to the ventrolateral muscles of the body wall (see below).

It was, for long, the case that once the myotome cells could be identified the remaining epithelial plate was termed the dermatome, its fate being described as forming the dermis of the skin. However, it is now clear that the epithelial plate continues to provide a significant source of myogenic precursor cells as it elongates into the body wall. Thus the detailed intimate relationship between the epithelial plate/dermatome, the generation of myogenic cells and the patterning of the epidermis of the skin is, as yet, by no means clear (see later). Studies describing a somitic contribution to the dermis, from the dermatome, localize it to the dermis over the epaxial muscles (Christ et al 1983^[1]), a much smaller distribution than the segmental portion of skin usually implied by this term.




The rate of somite formation has been estimated at approximately one pair every 3 hours (Menkes et al 1961; Chernoff & Lash 1981^[2]). The regularity of somite formation provides criteria for staging embryos; staging schemes have been developed both by Lash and Ostrovsky (1986^[3]) and by Ordahl (1993^[4]). Lash and Ostrovsky describe five stages, from somitomere identification (see above) to the production of an epithelial somite distinct from the presegmental plate. Ordahl notes that morphogenetic events occur in successive somites at approximately the same rate. He designates the somite most recently formed from the segmental plate (stage 5 of Lash & Ostrovsky) as stage I, the next most recent as stage II, etc. After the embryo forms an additional somite, the ages of the previously formed somites increase by one roman numeral. In this conceptualization of somitogenesis compaction occurs at stage I; epithelialization at stages II to III; formation of mesenchymal sclerotome cells from stage V; myotome formation at stage

VI; early migration of the ventrolateral lip of the epithelial plate and production of myotome cells can still be seen at stage X.



Differences have been identified in the fates of each of the six facets of a somite. Firstly the medial and lateral halves of the early epithelial somite have different origins and fates, later the cranial and caudal halves of the epithelial plate differ and finally the cranial and caudal halves of the sclerotome have different properties and fates. Experimental studies have shown that the precise developmental fates of these portions of the somite may be prescribed as the precursor cells ingress from the epiblast. Compaction and epithelialization may then shuffle these cells into their appropriate positions in the somite prior to migration.


Selleck and Stern (1991) have shown that the medial halves of somites are formed from cells migrating through the lateral portion of Hensen's node; the lateral halves derive from ingression through the primitive streak approximately 200 µm caudal to the node. The two somite halves do not seem to intermingle. The *medial half of the somite* produces both the *sclerotome* and the *myotome* (*epaxial musculature*); the *lateral half of the somite* provides the *hypaxial* and *limb musculature* (Ordahl & Le Douarin 1992; Ordahl 1993). (Interestingly the innervations of these muscle groups are provided by the posterior ramus of a spinal nerve for the epaxial muscles, and the ventral ramus for the hypaxial and limb muscles.) Of the other facets, the cranial portion of the epithelial plate is the site of origin of the myotome (see below). Differences in the craniocaudal fates of the portions of the somites have been studied in the development of the sclerotomes (see below).




Formation of the Vertebrae From Sclerotomes





The sclerotome forms from the ventromedial border of the epithelial somite. An *intrasegmental boundary* (fissure or cleft) appears within the *sclerotome* dividing it into loosely packed *cranial* and densely packed *caudal* halves; this boundary is initially filled with extracellular matrix and only few cells. The epithelial plate and later the myotome spans the two half-sclerotomes. The sclerotomal cells migrate towards the notochord which they surround, and with which they undergo a matrix-mediated interaction, differentiating chondrogenetically to form the cartilaginous precursor of the vertebral body. The perinotochordal sheath transiently expresses type II collagen which is believed to initiate type II collagen expression, and thereafter a chondrogenic fate, in those mesenchyme cells which contact it (see also development of the chondrocranium, p. 274). Each *vertebra* is formed by the combination of much of the *caudal half of one bilateral pair of sclerotomes with much of the cranial half of the next caudal pair of sclerotomes*. Their fusion around the notochord produces the blastemal centrum of the vertebra (3.132; see also 3.134). The mesenchyme adjoining the intrasegmental sclerotomic fissure now increases greatly in density forming a well-defined *perichordal disc*. This intervenes between the successive centra of two vertebrae and is the future annulus fibrosis of the intervertebral symphysis ('disc').


The boundary of the head and neck corresponds to the boundary between the 5th and 6th somites. The first true somite disappears early and somites 2–5 (occipital 1–4) fuse to form the

basioccipital bone (see, however, preoccipital somitomeres). The vertebrae are formed from the 6th somite caudally, C1 being formed by the caudal half of occipital somite 4 and the cranial half of cervical somite 1 (3.132 , 133 ). This shift of somite number and vertebral number accounts for the production of seven cervical vertebrae from eight cervical somites.

The basic pattern of a typical vertebra is initiated by this recombination of caudal and cranial sclerotome halves, followed by differential growth and sculpturing of the sclerotomal mesenchyme which encases the notochord and neural tube. This is the blastemal stage of vertebral development (3.134 ). As noted, the *centrum* encloses the notochord and lies ventral to the neural tube. From the dorsolateral angles of the centrum the neural arch curves to enclose the neural tube; from the zones of neurocentral confluence the arch comprises paired bilateral pedicles (ventrolaterally) and laminae (dorsolaterally) which coalesce in the midline dorsal to the neural tube. From the latter arises the anlage of the vertebral spine. On each side three further processes are delineated, projecting from the junction of pedicle and lamina cranially, caudally and laterally. The cranial and caudal projections are the blastemal *articular processes* (zygapophyses) and these become contiguous with reciprocal processes of adjacent vertebrae, their junctional zones the future zygapophyseal joints. The lateral projections are the true vertebral *transverse processes* (see below). Finally, growing anterolaterally from the ventral part of the pedicles, i.e. near the centrum, from the neighbouring perichordal disc, and, at most thoracic levels, with accessions from the next caudal adjacent pedicles, bilateral *costal processes* develop; these expand to meet the tips of the transverse processes. Note that the definitive vertebral *body* is compound, a median centrum and ventral expanded pedicle ends (bilateral) dorsolaterally.

In *cervical vertebrae* (3.134 ) the transverse process is dorsomedial to the foramen transversarium, while the costal process, corresponding to the head, neck and tubercle of a rib, limits the foramen ventrolaterally and dorsolaterally. The distal parts of these cervical costal processes do not normally develop; occasionally they do so in the case of the seventh cervical vertebra, even developing costovertebral joints. Such *cervical ribs* may reach the sternum (p. 268 ). In the thoracic region the *thoracic costal processes* attain their maximum length as *the ribs* (see below). The extent of the transverse and costal processes of each vertebra can be compared in (3.134 ).

The *type* of vertebra is specified very early in development. If a group of thoracic somites is transplanted to the cervical region, ribs will still develop (Kieny et al 1972 ; Goldstein & Kalcheim 1992 ). Interestingly it is the sclerotome which is restricted; the myotome will produce muscle characteristic of the new location. At present the exact contribution of the caudal and rostral parts of the sclerotomes to the neural arches, pedicles and laminae and articular and transverse processes are not yet entirely clear (Bagnall et al 1988 ; Goldstein & Kalcheim 1992 ); similarly the exact origin of the intervertebral disc has not been established.

Condensation of the sclerotomal mesenchyme around the notochord can be seen in stage 15 human embryos as can right and left neural processes. Chondrification begins at stage 17, initiating the cartilaginous stage (3.134 ). Each centrum chondrifies from one cartilage anlage

(Uththoff 1990^[4]). Each half of a neural arch is chondrified from a centre starting in its base and extending ventrally into the pedicles, to meet, expand and blend with the centrum, and dorsally into the laminae. By stage 23 there are 33 or 34 cartilaginous vertebrae; however, the spinous processes have not yet developed giving a general appearance of total spina bifida occulta. Fusion of the spines does not occur until the fourth month. The transverse and articular processes are chondrified in continuity with the neural arches; intervening zones of mesenchyme which do not become cartilage mark the sites of their interarticular (zygapophyseal) joints and the complex of costovertebral joints, and synovial cavities appear later in these.

In general the thoracic spine develops ahead of the cervical and lumbar spine; however, towards the end of the second month ossification commences in the cartilaginous vertebrae in a craniocaudal progression. After 16 weeks it has progressed to L5 and ossification of each additional vertebra occurs over a period of 2–3 weeks with S2 being ossified by 22 weeks. In most cases S1 can be located at the level of the top of the iliac crest (Budorick 1991^[5]). Further details of ossification are described elsewhere (p. 471^[6]).

Intervertebral Discs

Whereas the sclerotomal mesenchyme forming the body of the vertebrae replaces the notochordal tissue which it surrounds, between the developing vertebrae the notochord expands as localized aggregates of cells and matrix, thus forming the *nucleus pulposus* of the intervertebral disc (3.132^[7], 134^[8]). This nucleus is surrounded by the intermediate part of each *perichordal disc* which forms the annulus fibrosus and differentiates into an external laminated fibrous zone and an internal cuff around the nucleus pulposus. The inner zone contributes to the growth of the outer, and near the end of the second month of embryonic life it begins to merge with the notochordal tissue, being ultimately converted into fibrocartilage. After the sixth month of fetal life notochordal cells in the nucleus pulposus degenerate, being replaced by cells from the internal zone of the annulus fibrosus. This degeneration continues until the second decade of life, by which time all the notochordal cells have disappeared (p. 513^[9]). Thus, in the adult, notochordal vestiges are limited, at the most, to non-cellular matrix.

It is to be re-emphasized here that the original sclerotomes are coextensive with the individual *metameric body segments*, and that each sclerotomic fissure, perichordal disc, and finally the maturing intervertebral disc lies opposite the *centre* of each *fundamental body segment*. From this, it follows that the discs also correspond in level to (i.e. form the anterior boundary of) the intervertebral foramina, their contained mixed spinal nerves, ganglia, vessels and sheaths. Posteriorly, bounding the foramina are the capsules of the synovial interarticular (zygapophyseal) joints. Cranially and caudally lie the rims of the vertebral notches of adjacent vertebrae. Thus all the structures listed (and other associated ones) are often designated *segmental*, whereas because of their mode of development, vertebral bodies are designated *intersegmental*.

Ribs, Costal Cartilages and Sternum

Ribs

These develop from the costal processes of the primitive vertebral arches, extending between the myotomic muscle plates. The development of ribs is usually limited to the thoracic vertebrae although ribs can arise occasionally from the seventh cervical vertebra. In the thoracic region (3.134) costal processes grow laterally to form a series of *precartilaginous ribs*. The transverse processes grow laterally behind the vertebral ends of the costal processes, at first connected by mesenchyme which later becomes differentiated into the ligaments and other tissues of the costovertebral joints. The capitular costovertebral joints are similarly formed from mesenchyme between the proximal end of the costal processes and the perichordal disc, and adjacent neural arch derived parts of usually two (sometimes one) vertebral bodies. Ribs 1–7 (vertebrosternal) curve round the body wall to reach the developing sternal plates. Ribs 8–10 (vertebrochondral) are progressively more oblique and shorter, only reaching the costal cartilage of the rib above and contributing to the costal margin. Ribs 11 and 12 are free (floating), with cone-shaped terminal cartilages providing muscle attachments (see p. 541). In *lumbar vertebrae* (3.134) the costal processes do not develop distally, but their proximal parts become the 'transverse processes' of these vertebrae, whose morphologically *true* transverse processes may be represented by their accessory processes (p. 526). Occasionally, movable ribs may develop in association with the first lumbar vertebra. Only the upper two or three *sacral costal processes* usually develop significantly (3.134). They fuse into the lateral mass of the sacrum, forming its ventral part. The *coccygeal vertebrae* are apparently devoid of costal processes.

Sternum

This is formed from bilateral condensations of *somatopleuric mesenchyme* (Gumpel-Pinot 1984) immediately ventral to the primordia of the clavicles and ribs; these are termed the sternal plates. They are immediately ventral to the rudiments of the clavicles and ribs, but are independent of them in their formation. As the ribs lengthen, the sternal plates *chondrify* and move medially towards each other fusing across the midline in a craniocaudal direction. This forms the *manubrium sterni* and four *sternebrae* which form the sternal body with which the clavicles and upper seven pairs of costal cartilages establish contact. The *xiphoid process* develops as a caudal extension of the sternal body. Hypertrophy of the cartilage cells as a preliminary to ossification occurs opposite future intercostal spaces. The ossification and further growth of the sternum and ribs is described later (see 6.123, and p. 539).

Formation of the Axial Muscles From Myotomes

Myogenic determination factors, MyoD, myogenin, Myf 5 and herculin/MRF 4 can first be detected in the medial half of the somite as early as stage II (Ordahl 1993), several hours prior to the onset of myotome formation. The *myotome* is formed in the following manner: cells of the epithelial plate are mainly orientated perpendicular to the back; however, the cells have different orientations according to their positions: they are transversely orientated along the dorsomedial edge and longitudinally orientated within the cranial edge of the epithelial plate (3.131).

Myotome cells originate from the longitudinally orientated cells at the cranial edge of the epithelial plate. Individual cells are originally produced and anchored at the craniomedial corner of the epithelial plate. Subsequently each sends a process to the caudal edge of the plate where it forms a second anchor point. Thus myotome formation continues caudally along the dorsomedial edge and laterally along the cranial edge. Each mononucleated, myotome cell thus becomes very elongated perpendicular to the cells of the epithelial plate. Development of subsequent cells produces a triangular shape of the myotome in its early formation. The growing myotome first reaches the caudal somite border on the medial side and later the ventrolateral edge (Kaehn et al 1988^[4]). When the vertebral bodies form, the future intervertebral fissure divides the sclerotome into rostral and caudal halves leaving the myotome fibres spanning the intervertebral joints and foramina. Thus the myotome derived muscles are always in a position to move adjacent vertebrae relative to each other.

Myotome cells are all *postmitotic embryonic myoblasts*; they fuse to form syncytia later in development (see above) to produce the *epaxial* musculature, the skeletal muscle dorsal to the vertebrae (erector spinae). The normal development of these myoblasts requires the presence of the neural tube. It is also suggested that there is a possible interaction between precursor myotome cells and the medial neural crest cells which are commencing their migration at this time. The epaxial muscles are innervated by the dorsal ramus of each spinal nerve. The latter divides into its primary dorsal and ventral rami as it emerges from its intervertebral foramen (see above).

At much later stages *satellite cells* enter the myotome. Interestingly the development of endo-, peri- and epimysium in relation to the epaxial muscles has not been addressed; no population of connective tissue mesenchyme has yet been identified with this body region.




Ventrolateral Trunk Muscles

These are formed from the epithelial plate of the somite. After production of the myotome and the precursor myogenic cells of the limb, the remaining epithelial plate (and attached myotome) grows into the *flank somatopleuric mesenchyme*. At this stage the epithelial plate is still proliferating and producing myogenic precursor cells. The epithelial plate has a leading edge or process from which single cells or clusters of cells migrate in a ventral direction. It may be that these epithelial plate cells, which are in a more immature state of differentiation, act as *pioneer cells* for further cell movement (Jacob et al 1986^[4]). The previously segmented processes from adjacent epithelial plates form a *unified premuscular mass*. Both postmitotic myoblast cells and still dividing plate cells can be seen in the body wall; this may represent early and later forming myoblasts which will form heterokaryotic myotubes.


The *premuscular mass* subdivides into *abdominal muscle blastemata* for the *external* and *internal oblique* muscles, *transversus abdominis* and *rectus abdominis*. At this time the number of somatopleural fibroblasts situated within the muscle-forming zone increases, and myotubes can be first seen. Lastly, there is a ventral shift of the already separated muscle blastemata within the growing abdominal wall to their definitive positions. During this process muscle

differentiation continues and muscular connective tissue, tendons and aponeuroses develop.

The Diaphragm

This is a partition between the thoracic and abdominal cavities; it derives from a variety of mesenchymal populations. Ventrally the *septum transversum mesenchyme* anchors the diaphragm to the anterior abdominal wall where it attaches to the xiphisternum and costal margin. The central portion of the diaphragm is formed from *splanchnopleuric mesenchyme* which surrounds the oesophagus and inferior vena cava and coats the pleuroperitoneal membranes. Laterally the diaphragm derives from the *somatopleuric mesenchyme* which is excavated by extension of the secondary pleural cavities into the costodiaphragmatic recesses. *Somitic myocytes* from the ventrolateral edge of the epithelial plate of somites C3, 4 and 5 migrate into the lateral regions of the diaphragm including the somatopleuric part. The central region becomes tendinous. The posterior attachment of the diaphragm descends to lower and lower positions until at the end of the second month it is opposite T12 or L1 (3.135 , 136 ; see also p. 181 ).

Pelvic Floor

This consists of the *ligamentous supports of the cervix*, and the *pelvic* and *urogenital diaphragms*, and constitutes another partition which traverses the body cavity. The dimensions of the pelvic cavity are much smaller than those at the caudal end of the thorax and the pelvic diaphragm is thus a smaller structure. Because of the irregular shape of the innominate bones the pelvic outlet has two planes which are filled by muscular groups arranged at different levels and directions. There is little information available about pelvic floor development in the human. The striated muscle derives from the somitic epithelial plates in a similar manner to the ventrolateral body wall. The *puborectalis* muscle appears in 20–30 mm embryos, following opening of the anal membrane, and striated muscle fibres can be seen at 15 weeks (Bourdelat 1992 ). Also at this time the smooth muscle of the urethral sphincter can be seen.

Upper and Lower Ends of the Trunk

The upper and lower ends of the trunk are narrowed as a result of the development of axial structures cranially, and both axial and appendicular structures caudally. Cranially, the size of the ribs, the cervical pleura with its suprapleural membrane and the attached scalenus minimus muscle, together with the disposition of the other scaleni, create a narrow thoracic inlet which admits to the thorax only the contents of the root of the neck. Caudally, however, the pelvic outlet serves a dual function. It maintains the position of the pelvic organs and continence of the excretory organs by an arrangement of sphincter muscles. In addition, particularly with reference to the size of the human fetal head at term, the osteoligamentous boundary of the pelvic outlet, in the human, is relatively larger than that of all other quadrupeds; thus it requires a relatively extensive muscular and fibrous diaphragm.


Head

The head is composed of the skull surrounding the brain, and an outer covering of muscles, glands and skin. The skull has two distinct portions: that surrounding the brain and special sense organs—the *neurocranium*—and the lower face and jaws (also the palate, hyoid, epiglottis and larynx)—the *viscerocranium*. Each part derives from different mesenchymal populations and by different methods. The neurocranium develops from the *paraxial mesenchyme* in the head, i.e. the first five somites and the unsegmented somitomeres rostral to the first somite (Meier 1981^[1]), and from ectoderm via the *neural crest*. The basal portion of the skull is similar in structure and development to the vertebral column and is preformed in cartilage. The viscerocranium derives from ectoderm via invaginated head *neural crest* which streams into the developing arches forming all the connective tissue elements of the face. Bones of the viscerocranium form in the main from membranous ossification but there are cartilage models in each arch. The contribution of neural crest to the neurocranium in mammals is not yet clear, although it has been established in the chick that neural crest mesenchyme gives rise to the large bones lateral and dorsal to the brain by membranous ossification (Couly et al 1992^[2]). Lateral plate mesenchyme does not extend into the head (see p. 286^[3]).

Neurocranium


The bones of the skull (3.137^[4]) are developed in the mesenchyme which surrounds the cerebral vesicles but, before the osseous state is reached, the cranium passes through blastemal and cartilaginous stages like other parts of the skeleton. However, not all parts pass through a phase of chondrification; and hence the *chondrocranium* is incomplete, the remainder comprising the mesenchymatous, *blastemal desmocranium*. Most of the cranial vault and limited parts of its base are thus not preformed in cartilage. The mesenchymatous (membranous) and cartilaginous parts of the skull will, for convenience, be considered in sequence; they develop together and complement each other in forming the complete cranium, some of whose bones are composite structures derived from both sources. All elements, of course, pass first through a mesenchymatous phase (3.137^[5]).

The *blastemal skull* (desmocranium) begins to appear at the end of the first month as a condensation and thickening of the mesenchyme which surrounds the developing brain, forming localized masses which are the earliest distinguishable cranial elements. The first masses evident are in the occipital region, outlining the basilar (ventral) part of the occipital bone. These form an *occipital plate*, from which two extensions on each side grow laterally and spread to complete a foramen around each hypoglossal nerve. At the same time the mesenchymal condensation extends forwards, dorsal to the pharynx, to reach the primordium of the hypophysis, thus establishing the *clivus* of the cranial base and the *dorsum sellae* of the future sphenoid bone. Early in the second month it surrounds the developing stalk of the hypophysis and extends ventrally and rostrally between the two halves of the nasal cavity, where it forms the anlage of the ethmoid bone and of the nasal septum. The notochord traverses the ventral occipital plate


obliquely, being at first near its dorsal surface and then lying ventrally, where it comes into close relationship to the epithelium of the dorsal wall of the pharynx, being for a time fused with it. It then re-enters the cranial base and runs rostrally to end just caudal to the hypophysis (3.137A ).




During the fifth week bilateral *otocysts* (auditory vesicles) become enclosed within the *otic capsules*, which soon differentiate into dorsolateral *vestibular* and ventromedial *cochlear* parts, enveloping the primordia of the semicircular canals and the cochlea. Between these two regions the facial nerve lies in a deep groove. The otic capsules fuse with the lateral processes of the occipital plate, leaving a wide hiatus through which the internal jugular vein and the glossopharyngeal, vagus and accessory nerves pass. At this stage the mesenchyme around the developing hypophyseal stalk, which is forming the rudiment of the postsphenoid part of the sphenoid bone, spreads out laterally to form the future greater wings of this element. Smaller processes rostral to this indicate the sites of the lesser wings of the sphenoid, while other condensations reach the sides of the nasal cavity and also blend with the still mesenchymatous septum.

Basal Regions of the Skull


These are, in mammals, initially preformed in cartilage (3.137 ). This occurs primarily in three regions:

- caudally, in relation to the notochord
- intermediately, in relation to the hypophysis
- rostrally, between the orbits and the nasal cavities.




These may be named *parachordal*, *hypophyseal* and *interorbitonasal* regions. The *parachordal cartilage* is developed from paraxial mesenchyme related to the cranial end of the notochord and the first five (occipital) somites; caudally it exhibits traces of four primitive segments separated by the roots of the hypoglossal nerves. It is notable that the region of fusion between the rostral part of the occipital bones and the portion of the parachordal plate that is of somitomeric origin corresponds to the spheno-occipital synchondrosis, which is the site of growth for up to 20 years of age. The *otic capsule* is formed from three different sources (identified in the chick): the first somite, a portion of paraxial mesenchyme and neural crest mesenchyme (Couly et al 1992 .




The *hypophyseal cartilage* ossifies to form the *postsphenoid part* of the *sphenoid bone*; it derives from both paraxial mesenchyme and neural crest in the chick (Couly 1992  (see also 3.101 ). The paraxial mesenchyme contributes to the caudal part of the sella turcica, forming each side of the rostral end of the notochord, whereas the neural crest forms the more rostral portion of the sella turcica, and the region termed by Couly et al (1992  the *prechordal skeleton*. The interorbitonasal cartilage is perhaps to be equated with the trabeculae cranii of lower vertebrates and is usually known as the trabecular cartilage, which is a bilateral structure developing from two centres of chondrification. The *trabeculae cranii* and the *ethmoid* complex are of neural

crest origin.

In the human embryo cranial chondrification begins in the second month; cartilaginous foci first appear in the occipital plate, one on each side of the notochord (parachordal cartilages); these later fuse at the end of the seventh week surrounding the notochord, whose oblique transit through the region has been mentioned (3.137A ). The cartilaginous posterior part of the sphenoid is formed from two hypophyseal centres, flanking the stalk of the hypophysis and uniting at first behind, then in front, enclosing a *craniopharyngeal canal* containing the hypophyseal diverticulum. The canal is usually obliterated by the third month; its association with the derivation of the anterior lobe of the hypophysis from the pharyngeal diverticulum of Rathke has been denied.



The otic capsules, presphenoid, bases of the greater wings and lesser wings of the sphenoid, and finally the nasal capsules, in turn become chondrified. The whole nasal capsule is well developed by the end of the third month, consisting of a common median septal part (sometimes initially termed the *interorbitonasal septum*) and two lateral regions. The free caudal borders of the latter incurve to form the interior nasal conchae, which ossify during the fifth month and become separate elements. Posteriorly each lateral part of the nasal capsule becomes ossified as the ethmoidal labyrinth, bearing on its medial surface ridges the future middle and superior conchae. Part of the rest of the capsule remains cartilaginous as the septal and alar cartilages of the nose; part is replaced by the mesenchymatous vomer and nasal bones.

The ventral surface of the chondrocranium is associated with the cartilages of the pharyngeal arches, the development of which will be considered later (see p. 275  et seq). The bones of the cranial base which are thus preformed in cartilage are the occipital (excepting the upper part of its squama), the petromastoid part of the temporal, the body, lesser wings and roots of the greater wings of the sphenoid, and the ethmoid. These constitute the cartilaginous part of the neurocranium. To summarize, therefore, the base of the skull—except for the orbital plates of the frontal and the lateral parts of the greater sphenoidal wings—is preformed in cartilage (3.137 , see also 3.101 ).

Specification of the pattern of the base of the skull may be caused by an epithelial/mesenchymal interaction involving the overlying neural tube. Thorogood (1988 ) proposed a 'flypaper model' of development for the cartilaginous neurocranium. The basal aspect of the neuroectoderm transiently expresses type II collagen around the olfactory regions, around the optic cups prior to and during invagination of the lens, around the otic vesicles, and on the ventrolateral surfaces of the diencephalon, mesencephalon and rhombencephalon (Thorogood 1988 ). The notochord also expresses type II collagen in its perichordal sheath. Some time later, after the neural expression of type II collagen has ceased, mesenchyme adjacent to the regions described above commences synthesis of type II collagen and begins differentiation into chondrocytes. Thorogood reasoned that the transient expression of type II collagen in the basal lamina of the neural epithelium causes localized arrest of cell migration of those mesenchyme cells which touch it, regardless of origin. (A similar mechanism is seen in the notochordal–sclerotome interaction; see p. 265 .) At such sites cells accumulate and undergo a matrix-mediated interaction with the neural



epithelium and differentiate along a chondrogenic lineage. Thus the pattern in which the cells are trapped, epigenetically determines the form of the chondrocranium. This has evolutionary implications, as slight alterations in the expression of the type II collagen by the neuroepithelium could have profound effects on the shape and form of the chondrocranium and on the whole skull, because the blastemal skull must connect to the plan initiated by the chondrocranium. This model could perhaps account for the diversity of skull shape seen in the vertebrates.


Ossification commences before the chondrocranium has fully developed, and as this change extends, bone overtakes cartilage until little of the chondrocranium remains. However, parts of it still exist at birth and small regions remain cartilaginous in the adult skull. At birth unossified chondrocranium still persists at:

- the alae, lateral nasal and septum of the nose
- the sphenothmoidal junction (p. 551 )
- the sphenothoccipital and sphenopetrous junctions (p. 551 )
- the apex of the petrous bone (foramen lacerum)
- and also between ossifying elements of the sphenoid bone and between elements of the occipital bone.

Most of these regions function as growth cartilages. For further development of these areas and cranial bones in general see Section 6, Skeletal system.

Vault or Upper Regions of the Skull

These first appear about the thirtieth day; they consist of curved plates of mesenchyme at the sides of the skull and gradually extend cranially to blend with each other; they also extend towards and reach the base of the skull, which will become part of the chondrocranium. The mesenchymatous (membranous) neurocranium, corresponding to the cranial vault, is not preformed in cartilage. Its elements, frequently described as dermal bones (p. 548 ) , are the frontal bones, the parietals, the squamous parts of the temporal bones and the upper (interparietal) part of the occipital squama. It is now believed that the frontal, parietal and squamosal bones are formed from neural crest. Also the sutures of the calvarium and facial bones are made up of crest cells (Couly et al 1992 ) .

There is a close association between the developing meninges, particularly the dura mater, and the calvarial bones. The dermal bones are formed by the initiation of a wave of osteodifferentiation moving radially from ossification centres within the desmocranial mesenchyme. When adjacent bones meet, proliferation of the osteogenic front ceases and sutures are induced to form. Once sutures are formed and the fibrous desmocranium is replaced by mineralized bone, a second phase of development occurs in which growth of the cranial bones occurs at the sutural margins (Opperman et al 1993 ) . Such growth forms most of the calvaria.

Opperman et al (1993^[1]) have demonstrated that transplants of sutures in which the fetal dura mater is left intact results in a continuous fibrous suture between developing vault bones, whereas in transplants in which the fetal dura mater is removed bony fusion occurs. This interaction of underlying dura mater with the developing calvarial bones has been demonstrated experimentally, showing that the dura not only promotes the position and maintenance of sutures, but also that dura can repattern both the reappearance and position of the bones and sutures of the cranial vault after removal of the calvaria in the neonate.

At the site of a developing suture the osteogenic fronts of two adjacent bones meet and overlap. Initially there is a highly cellular suture blastema between the bones which later becomes more dense and acellular. In the mature suture a narrow overlap of compact bone contains a dense, narrow band of cells continuous with the periosteum.

Musculature Associated with the Neurocranium

Most of the striated musculature of the head is formed during development of the viscerocranium when muscle masses, particularly from the second pharyngeal arch, migrate to cover parts of the neurocranium (see p. 284^[1]). However, two further sources of muscle provide myoblasts for the external ocular muscles and the tongue.

Extrinsic Ocular Muscles

All extrinsic ocular muscles derive from prechordal mesenchyme which lies at the rostral tip of the notochordal process and remains mesenchymal after the notochordal process becomes epithelial and gains a basal lamina (3.148^[1]). In early embryos prechordal mesenchyme migrates laterally towards the paraxial mesenchyme (p. 144^[1]). Its early myogenic properties in the head can be demonstrated by chimeric recombination, and further, if transplanted into limb buds it is able to develop into muscle tissue.

Early embryos develop bilateral cavities in the head, previously described as preotic somites. The walls of the premandibular head cavities are lined by flat or cylindrical cells which do not exhibit the characteristics of a germinal epithelium like the epithelial plate of the somite; also there is no basal lamina around the head cavities. As the oculomotor nerve grows down to the level of the head cavity a condensation of premuscle cells appears at the ventrolateral side of the head cavity. Later the head cavities are filled with ingrowing mesenchyme. The premuscular mass subdivides into the blastemata of the different muscles supplied by the oculomotor nerve (Wachtler & Jacob 1986^[1]). Similar events occur with respect to the intermediate head cavity (trochlear nerve and superior oblique muscle), and the caudal head cavity (abducent nerve and lateral rectus muscle).

There is no doubt that the head cavities are formed by a mesenchymal/epithelial shift similar to that seen in the somites. However, the epithelial plate of the somite is a germinal centre which produces postmitotic myoblasts destined for epaxial regions, and migratory premitotic myoblasts

destined for the limbs and body wall. The head cavities may serve a similar purpose if a mesenchyme/epithelial shift is part of a maturation process for putative myoblasts; however, it may not need to provide a centre for cell replication: premitotic myoblasts differentiated directly from the prechordal mesenchyme may form the premuscular masses.

Muscles of the Tongue

This development appears to be similar to the development of the muscles of the limb. Single, premitotic cells detach from the ventrolateral portion of the occipital somites and migrate to their ultimate positions (Wachtler & Jacob 1986^[1]) (3.131^[2], 148^[3]). The connective tissue surrounding these muscles is derived from neural crest cells (Noden 1983^[4]).

Viscerocranium

The development of the viscerocranium is very complex. It involves the migration and interaction of epithelial populations derived from: the neural folds, surface ectoderm and endoderm; mesenchymal populations from the mesencephalic, metencephalic and myelencephalic neural crest, paraxial mesenchyme and angiogenic mesenchyme; and neural populations from the neural tube, neural crest and ectodermal placodes. Generally, the more rostral structures, i.e. face, palate, buccal cavity and nasal cavity, derive entirely from ectodermal populations (both epithelium and mesenchyme—via neural crest), whereas the caudal and related structures, i.e. pharynx and larynx, are derived from ectoderm, neural crest and interactions with endoderm.

The development of the face and neck is intimately related to the development of the brain and special sense organs; the reader is advised to refer to the development of the neural crest on pages 147^[5] and 220^[6], and of the head, page 157^[7], and to 3.100^[8], 101^[9], 148^[10].

All of the structures which give rise to the face and neck are segmentally organized during development; the hindbrain displays rhombomeres, the ectoderm—ectomeres and the paraxial mesenchyme—somitomeres. The overall segmentation of this region is related to the expression of axial genes in the head which have been conserved throughout evolution.

Vertebral Pharyngeal Apparatus

In all vertebrate embryos, after head fold formation the *stomodeum*, or primitive mouth, is bounded cranially by the projecting forebrain and caudally by the cardiac prominence (3.138^[11]). The mandibular region and the whole of the neck, which will subsequently intervene between mouth and developing thorax, are absent, but will be formed by the appearance and modification of six paired *branchial (gill) arches*, which develop in the lateral aspects of the head adjacent to the hindbrain (3.139^[12], 140^[13], 141^[14]). In the earliest vertebrates which were jawless (*Agnathia*), the branchial arches were a uniform series of bars behind the gill clefts; but long before the evolution of the terrestrial vertebrates, remarkable adaptations had occurred in them.

Structures commonly regarded as the first pair of arches became the jaws, upper and lower, of the jaw-bearing vertebrates (*Gnathostomata*), including most fish; they are, therefore, usually named the *mandibular arches*. (The term 'mandibular arch' is widely used but not entirely appropriate because of the numerous maxillofacial, nasal, otic and palatopharyngeal derivatives from its dorsal end.) It should, however, be noted that since this early identification, strong evidence has accumulated that, at least, a pair of *premandibular arches* existed and have become adapted as the *trabeculae cranii* of subsequent vertebrate embryos. These are probably represented by the interorbitonasal cartilage of the human embryo (see p. 271 [\[1\]](#)) which forms a branchial element in the chondrocranium. The next (*postmandibular*) arch in the series is the *hyoid arch*; its skeletal derivatives form the varied hyoid elements present in all vertebrates with jaws. The most dorsal of the latter, the *hyomandibula*, is already present in cartilaginous fish as a strut between the skull and the primitive jaw joint, thereby reducing the cleft between the mandibular and hyoid arches to a small opening, the *spiracle*. The interesting further evolution of this region in land animals in connection with the auditory apparatus has been considered (p. 263 [\[2\]](#)). The hyoid arch also contributes to the formation of a gill cover, or *operculum*, in bony fish, and the remaining arches persist as the supports of the gill apparatus.

At first the arches produce rounded ridge-like prominences both of the overlying ectoderm and of the endodermal lining of the lateral walls and floor of the pharynx. In the furrows between these prominences the ectoderm and endoderm are in virtual contact. The thin membranes so formed break down permanently in gill-breathing vertebrates, transiently in reptile embryos, but persist in the tetrapods, in which open channels or 'true clefts' are not formed. However, the external *branchial grooves* which correspond to them are frequently, less appropriately, called *branchial clefts* and their internal counterparts are the *pharyngeal sacs* or *pouches*.

In gill-breathing vertebrates the exchange of respiratory gases is directly from solution in water to solution in blood. From the cranial end (arterial, but carrying deoxygenated blood) of the heart emerge two *ventral aortae* which traverse the ventral pharyngeal wall, sending branches curving dorsally into the branchial arches, where they feed capillary plexuses in the gills. These are drained by corresponding arteries, which join two *dorsal aortae* supplying the general circulation. As water is taken in through the mouth and passed back through the gill clefts, its dissolved oxygen diffuses through the pharyngeal endoderm and endothelium of the gills to reach the blood, carbon dioxide diffusing out of the latter into the water. This intimate relationship between the developing mouth, branchial apparatus and heart in water-breathing vertebrates is repeated in the embryos of their tetrapod descendants, but with many modifications necessary to changed respiratory function.

Although a description of the branchial apparatus is appropriate for water-breathing vertebrates, the application of this terminology to mammalian embryos is by no means universal. O'Rahilly and Muller (1992 [\[3\]](#)) consider the term *branchial* to be inappropriate for mammalian embryos. Similarly the term *visceral*, used synonymously to describe the arches, has been questioned by Noden (1991 [\[4\]](#)) who notes that 'visceral' suggests a primary relation between the arches and the internal pharyngeal tube that obscures the somatic function of most of the tissue within the arches. The region of the embryo containing the rostral foregut, surrounded by mesenchyme and ectoderm, constitutes the embryonic pharynx; the stage of development at which the arches are

prominent has been termed the *pharyngula stage* (see p. 100📖). However, the appellation *pharyngeal* arches is also problematical as the first arch which forms most of the face is in the main composed of ectoderm alone, both on the outer and inner surfaces and within the arch (neural crest mesenchyme). Thus the first arch is technically not a pharyngeal structure, unlike the subsequent caudal arches which are composed of ectoderm externally, endoderm of the pharynx internally and neural crest mesenchyme within the arches. This difference in origin of the first and subsequent arches is related to the evolution of the head and skull (see p. 287📖). For the purposes of the description of human embryology the term *pharyngeal* arches will be used; however, comparison with other species will involve the term branchial.

A Typical Pharyngeal Arch

Generally each pharyngeal arch consists of an epithelial covering exteriorly and a mesenchymal core interiorly (3.139👁, 140👁, 141👁). The epithelium may be ectodermal entirely (as in the first arch), or ectoderm covering the external aspect of the arch and endoderm covering the internal aspect of the arch (as in the remaining arches). The mesenchyme within each arch derives from neural crest, paraxial and angiogenic mesenchymal populations. The motor and sensory roots of a cranial nerve are associated with the epithelium and mesenchyme of each arch.

From these disparate cell populations each arch develops:

- region-specific *epithelial structures*
- a *skeletal element* from the neural crest mesenchyme
- associated *striated muscle* from the paraxial mesenchyme
- an *arch artery* from the angiogenic mesenchyme
- *motor and sensory nerves* specific to the arch.

The epithelia covering each arch is patterned by the underlying mesenchyme. Such patterning is specific for individual arches and results in such diverse specializations as: keratinized stratified squamous epithelium, hair, sweat, sebaceous and ceruminous glands; pseudostratified ciliated columnar epithelium, teeth, salivary, mucous and lacrimal glands; the epithelia of glands such as the thyroid, parathyroids, thymus; and of the lymphoid tissues in the oro- and nasopharynx.

The skeletal element is formed from neural crest mesenchyme which condenses and subsequently chondrifies either wholly or in part of its length; if this change is complete the element extends dorsally until it comes into contact with the mesenchymatous cranial base lateral to the hindbrain. The arch cartilage, entirely or in part, may remain as cartilage, undergo endochondral ossification, be replaced completely by intramembranous ossification, or become ligamentous. Neural crest also gives rise to the *ligaments, tendons* and *connective tissue* in the arches and the *dermis* underlying the skin. Generally the neural crest controls the pattern of development of the arches and is itself programmed by the expression of *Hox* genes in the

hindbrain (see p. 227📖).

The striated muscle of each arch, sometimes termed *branchial musculature* to denote its origin, derives from the unsegmented paraxial mesenchyme of the head, the somitomeres (see pp. 154📖, 285📖); the myoblasts may migrate great distances and lose connection with the skeletal elements in the arches which cease their original respiratory function. The identities of these muscle masses, where they assume new functions, can nevertheless be inferred by reference to their nerve supply.

An arch artery develops in each arch either by vasculogenesis, where angioblastic mesenchyme migrates into a region and initiates vessel development in situ, or by angiogenesis, where vessels develop by sprouting from the endothelium of pre-existing vessels (see p. 299📖). The paired arch arteries arise from the truncus arteriosus and pass laterally each side of the pharynx to join the dorsal aortae.




Nerves arise from the adjacent hindbrain (3.96👁️, 112👁️). They pass directly into the arches, which are ventral to it, by two methods. *Motor nerves* grow out from the basal plate of the midbrain and hindbrain to innervate the striated muscle in the arches. Generally these nerves are termed *special branchial efferent* noting their innervation of branchial musculature. *Sensory nerves* extend from cranial sensory ganglia which are derived in part from neural crest cells and in part from ectodermal placodes (see p. 237📖); they convey *general* and *special somatic afferent* axons. Within the arch a mixed nerve typically runs along the rostral border and is hence described as *post-trematic*, because it is behind or caudal to the cleft or trema rostral to the arch. A sensory branch from this principal post-trematic nerve passes to the immediately rostral arch where it runs close to the caudal border; it is thus *pretrematic* with respect to the cleft caudal to it (3.139👁️). In the human embryo the pre- and post-trematic nerves cannot be identified with certainty.





Development of the Pharyngeal Arches




The human circumoral *first pharyngeal arch* (3.138👁️, 139👁️) consists, on each side, of two main regions: a *ventral part* or *mandibular prominence* and a *dorsal part* or *maxillary prominence*. Each mandibular prominence, first seen at stage 10 (22 postovulatory days), grows ventromedially in the floor of the pharynx to meet its fellow in the midline, being situated between the primitive mouth and the cardiac (pericardial) prominence. The maxillary prominences are not seen until stage 13; their enlargement coincides with proliferation of neural crest mesenchyme between the ectoderm and prosencephalon forming the frontonasal prominence (see below). The enlargement of the first arch is particularly rostral to the site of the buccopharyngeal membrane; thus inner and outer aspects of this arch are covered with ectoderm. The *second* or *hyoid arches*, seen from stage 11, are caudal to the maxillomandibular; they similarly grow ventrally to meet and fuse in the midline. The *third arches* are seen at stage 12 (26 days) and the *fourth arches* by stage 13 (28 days); the latter especially are not prominent, being largely sunk in a depression produced by the caudal overlapping of the hyoid arch. The

fifth and *sixth* arches cannot be recognized externally and can only be identified by the arrangement of the mesenchyme and by slight projections into the pharynx.

The First Pharyngeal Arch

The first pharyngeal arch is sufficiently different, both in its structure and development, from the subsequent caudal arches for its separate examination. Unlike the other arches it possesses dorsal and ventral prominences, appearing C-shaped in lateral view (see 3.142 ). The dorsal (maxillary) prominences interact with ectodermal epithelia and neural crest mesenchyme of the frontonasal prominence, and generally form more extensive skeletal structures than the other arches (see p. 284 ); particularly, these skeletal elements fuse with the chondrocranium. The first arch is completely clothed with ectoderm unlike the caudal arches which are dependent on the proximity of pharyngeal endoderm for their development. The ectoderm originates (in the 3-somite chick) from a territory lateral to the mesencephalic neural folds (see 3.145 ). The mesencephalic folds themselves give rise to both the ectodermal placodal cells and neural crest cells which contribute to the trigeminal ganglion, and the mesenchymal population which streams into the mandibular and maxillary prominences.





The first arch contains on each side a dorsal and ventral cartilage. The former represents the *palatopterygoquadrate* bar, a prominent element in earlier vertebrates forming part of the upper jaw but much reduced in mammals. In human embryos its early appearance seems transient and its contribution to some permanent cranial structures, such as the maxilla, is uncertain (however, see below). The *ventral cartilage* (of Meckel, 3.143 ) extends from the developing otic capsule into the mandibular prominence, meeting its fellow at its ventral end. The dorsal end of Meckel's cartilage becomes separated, and was often held to form the rudiments of both *malleus* and *incus*. However, there is strong palaeontological (Romer 1970 ) and comparative anatomical (Shute 1956 ) evidence that the incus is, in part, to be regarded as a homologue of the *quadrate bone* of reptiles, and it is therefore probably more correctly regarded as a derivative of the palatopterygoquadrate cartilage. This cartilage may also contribute to the ala major of the sphenoid bone and the roots of its pterygoid plates. Beyond the rudiment of the malleus, the intermediate part of Meckel's cartilage disappears, but its sheath persists as the *anterior malleolar* and *sphenomandibular ligaments*. The ventral part, much the largest, is enveloped by the developing mesenchymatous mandible (p. 577 ); a small fraction of this, extending from the mental foramen almost to the site of the future symphysis, probably becomes ossified from invading mandibular tissue, into which it is incorporated, while the remainder of the cartilage is ultimately absorbed.






The cells which give rise to the muscle of the first arch arise from the paraxial mesenchyme localized to somitomeres 2 and 3 (Trainor et al 1994 ) (p. 285 ). The muscle mass of the mandibular part of the first arch forms the *tensor tympani*, *tensor veli palatini* and the *masticatory muscles*, including *mylohyoid* and the *anterior belly of digastric* (3.144 ). The tensor tympani retains its connection with the skeletal element of the arch through its attachments to the malleus, and the tensor veli palatini to the base of the medial pterygoid process, which may be derived from the dorsal cartilage of the first arch, but the masticatory

muscles transfer to the mandible, a dermal bone.

All these muscles are supplied by the mandibular nerve, the mixed 'post-trigeminal' nerve of the first arch.

Face

While the mandibular prominences are invading the floor of the pharynx, mesencephalic neural crest cells migrate rostrally and laterally between the prosencephalic neuroepithelium and the surface ectoderm to form the extensive *frontonasal prominence*. During the fifth week the sites of the *olfactory* or *nasal placodes* are established ventrolateral to the frontonasal prominence, dividing the latter, on each side, into *medial* and *lateral nasal prominences* or folds; the olfactory placodes originate from the neural folds (p. 222 ). The placodes are at first widely separated and coplanar with the surface ectoderm but, as the nasal prominences develop, they soon become depressed to form the *olfactory pits* (nasal sacs). The olfactory placodes are the anlage of the olfactory and vomeronasal epithelia, which derive from the rostral neural folds; these folds also give rise to the respiratory epithelia of the nasal cavity (see 3.145 ). The lateral nasal prominences are the more evident (3.142 , 3.146B ), but the medial nasal prominences, still separated by the median remainder of the frontonasal field, project caudally beyond the former. Extensions of mesenchyme from the medial prominence into the roof of the stomodeum proliferate to form the *premaxillary* fields. Each nasal sac has a ventral fold from which develops an epithelial *nasal fin* passing caudally to fuse with the stomodeal roof.











While these changes are progressing a somewhat triangular elevation swells ventrally from the cranial aspect of the dorsal region of each mandibular prominence. This is the *maxillary prominence*, and like the frontonasal prominence it consists of proliferating neural crest mesenchyme covered by ectoderm. Each maxillary prominence grows in a ventral direction and fuses with the lateral nasal prominence, the two being at first separated by a *nasomaxillary groove* (*naso-optic furrow*) (3.142 ; Streeter 1948 ). The opposed margins of the lateral nasal and maxillary prominences growing together thus establish continuity between the side of the future nose and the cheek (3.142 ). The ectoderm along the boundary between them does not entirely disappear; it gives rise to a solid cellular rod, which at first develops as a linear surface elevation, the *nasolacrimal ridge*, and then sinks into the mesenchyme (Politzer 1952 ). Its caudal end proliferates to connect with the caudal part of the lateral nasal wall, while its cranial extremity later connects with the developing conjunctival sac. The solid rod becomes canalized to form the *nasolacrimal duct* (3.146B ).

(It should be noted that the epithelial folds and elevations due to loci of proliferation of underlying mesenchyme were long termed processes. The International Nomenclature Committee felt that this was not entirely appropriate and their revised term 'prominence' has been adopted here. Both terms are used in the literature.)



The relatively wide primitive mouth or *stomodeal fissure* is progressively reduced, and the epithelial and connective tissues of the cheek enlarged, by fusion between the adjacent surfaces



of the mandibular and maxillary prominences. This proceeds from the para-otic region to the angle of the definitive *oral fissure*.



Nasal Cavity




The rounded apex of the triangular maxillary prominence extends beyond the lateral nasal prominence, crossing the caudal end of the olfactory pit to meet and fuse with the *premaxillary elevation* developing at the extremity of the frontonasal field. This closes off the lower or caudal edge of the olfactory pit, the upper part of the opening of which is thus defined as the primitive *external naris*. The growth of the surrounding mesenchyme leads to a deepening of the pit to become a primitive nasal cavity, or *nasal sac*, the epithelial wall of which, in the dorsocaudal part of its extent, the nasal fin, retains contiguity with the epithelium of the stomodeal roof. This contact area becomes progressively greater as growth continues, and the nasal fin is eroded, ultimately forming a thin layer, the *oronasal membrane* (3.146A ) , which also disappears later. Thereafter, the primitive nasal cavity communicates with the stomodeum through a primitive *internal naris* (*choana*), which is at this stage still well forward or ventrally situated in the stomodeal roof (Warbrick 1960 ). By these changes a new cranial boundary is set for the oral opening, consisting of the fused premaxillary and maxillary regions. This is the future upper lip, but it has not yet become separated from the deeper tissues which will form the maxillary alveolus. At the same time the nasal cavity acquires a floor through the fusion of the nasal prominences and the maxillary prominences. At this stage the two external nares are still widely separated by an area derived from the frontonasal field, but this separation becomes reduced by the fusion of the premaxillary mesenchyme from the two sides. According to some investigators the mesenchyme of the maxillary prominences invades the premaxillary regions, the mesenchyme of which is said to become buried, to form later the premaxilla or os incisivum (p. 574 ; Boyd 1933 ; Baxter 1953 ). The maxillary mesenchyme is thus considered by some to contribute substantially to the formation of the *philtrum* of the upper lip, thus accounting for its maxillary innervation. Others, however, maintain that the philtrum is derived wholly from premaxillary tissue (Keith 1948 ; King 1954 ; Warbrick 1960 ; Wood et al 1967  (see also 3.100 ). The maxillary nerve primarily innervates the maxillary mesenchyme but apparently extends later into the territory of the frontonasal prominence. It should be added that some workers deny that sensory nerve distribution is a reliable guide to migration of mesenchyme in the case of the maxillary prominence.

Palate



Once the primitive nasal cavities are defined the ventral part of the roof of the oral cavity can be regarded as the *primitive palate* (*median palatine prominence*; 3.146A ). It is formed by the premaxillary regions and maxillary prominences, which become confluent and establish continuity with the thick median *nasal septal prominence* (*primitive nasal septum*). As the head grows in size, the region of mesenchyme between the forebrain and oral cavity increases greatly by proliferation and the nasal cavities deepen, extending towards the forebrain. Simultaneously they also extend dorsally from the primitive choanae as two narrow and deep grooves in the oral roof (3.146 ) which are separated by a partition. The grooves and the partition deepen together, and the latter becomes the *nasal septum*, continuous rostrally with the *primitive nasal*





septum (3.146B ). The broad dorsocaudal border of the nasal septum is at first in contact with the dorsum of the developing tongue (3.146B ), the right and left nasal cavities still communicating freely with the mouth except where the nasal floor is already established ventrally by the primitive palate.

During Stage 17 (41 days) the internal aspects of the maxillary prominences produce *palatine processes* (*shelves*), which grow towards the midline but are for some time separated from each other by the tongue. At this stage the roof of the oral cavity projects ventrally beyond its floor and the tip of the developing tongue actually lies in contact with the cranial (superior) surface of the primitive palate. A coronal section dorsal to this shows the maxillary palatine processes contiguous with the sides of the tongue and bent into a vertical position on each side of it (3.146B ). With further growth, the mandibular region and the tongue are carried forwards (ventrally), and the lingual tip passes round to the caudal surface of the primitive palate. At stage 23 (56–57 days) the palatine processes rapidly elevate, assuming a horizontal position which allows them to grow towards each other and thus to fuse (3.146C ); this occurs from before backwards.



The change of position occurs very rapidly caused by the progressive region specific synthesis and accumulation of hyaluronic acid within the palatal process mesenchyme. The hyaluronic acid will bind up to 10 times its own weight of water, thus causing swelling and expansion of the palatal shelves. This process is further aided by the alignment of collagen fibrils and palatal mesenchymal cells (the latter contract in response to acetylcholine and serotonin which they secrete thus regulating the elevation of the shelves), and by the epithelium which restrains the swelling. Once these forces are in concert and exceed the resistance factors, the palatal shelves will mechanically elevate. Such elevation occurs at a time of craniofacial growth when there is constant growth in head height but almost no growth in head width. This latter factor is important: if palatal shelf elevation is delayed so that they elevate in a period of growth in facial width, the unfused processes are unable to touch physically and cleft palate may result. Other factors affecting palatal closure are the growth in length of the first arch cartilage (Meckel's) which allows the tongue to lower into the developing mandible. Further, the change in position of the maxilla relative to the anterior cranial base, which is maintained at about 84° during weeks 9 and 10, has the effect of lifting the head and upper jaw upwards from the mandible so permitting withdrawal of the tongue from between the palatal shelves and creating space for them to elevate. Mouth opening, tongue protrusion and hiccup movements have also been noted at this time; these movements and their associated pressure changes may assist palatal shelf elevation (Ferguson 1977 , 1990 , 1993 ). Generally in female embryos palatal shelf elevation occurs 7 days later than in males, making congenital cleft palate more likely in female embryos. After elevation the palatine processes grow medially along the inferior borders of the primitive choanae, uniting with them and with the margins of the median palatine prominence, except over a small area in the midline where a *nasopalatine canal* maintains connection between the nasal and oral cavities for some time and marks the future position of the incisive fossa. (The plates which form the early (primitive) palate are sometimes known as *median* palatine processes, the maxillary contributions being then named the *lateral* palatine processes.)

As the medial borders of the maxillary palatine processes fuse together, fusing also with the free

border of the nasal septum, the nasal and oral cavities are progressively separated and the tongue is excluded from the former. The nasal cavities are thus extended dorsally and the choanae reach their final position, leaving the caudal edge of the nasal septum free in about its dorsal quarter as the partition between them. Slightly later the dorsomedial extremities of the palatine processes, which extend dorsally beyond the choanae, fuse together rostrocaudally to form the future epithelia and connective tissues of the soft palate (3.146C ). There is later an upgrowth of myogenic mesenchyme from the third and, probably, other pharyngeal arches into the palate and around the caudal margins of the auditory tube, along a line corresponding in the final state to the palatopharyngeal arches (Baxter 1953 .

On each side of the nasal septum, in a ventral or anterior position just above the primitive palate, placodal ectoderm is invaginated to form a pair of small diverticula, which extend dorsally and cranially into the septum. These are vestiges of the *vomeronasal organ* (3.146C , whose openings are close to the junction between the two premaxillae and the maxillae; they are always rudimentary in mankind, but are well-developed auxiliary olfactory organs in many vertebrates (pp. 1225 , 1321 ). For bibliographies in the field of facial development consult Latham (1973 .


Facial Epithelium

The external ectoderm over the mandibular prominences becomes the skin of the face (3.147 ) , and it also takes part in forming the tragus of the auricle (p. 1368 ). Its surface facial contribution is roughly triangular; the apex includes the tragus, the upper border extending to the lateral angle of the mouth and free border of lower lip; its lower border curves to follow the principal submandibular flexure line of the neck. The surface facial contribution of the maxillary prominence extends from the supratragic point to the lateral angles of eye and mouth, includes the lower eyelid and follows the paranasal line of the nasolacrimal duct, finally including a controversial amount of the upper lip.

The ectoderm on the arched, circumoral borders of both the mandibular and maxillary prominences, including the premaxilla medially, thickens along two curved parallel arches. The external thickening is the *labiogingival* or *vestibular lamina*, and the internal the *dental lamina*. The labiogingival lamina invades the subjacent mesenchyme and subsequently breaks down to form a sulcus (the vestibule) which separates the lower and upper lips from their adjacent gums. Within the mandibular prominence, the gum is separated from the tongue by the *linguogingival groove*. The dental lamina denote the sites of development of the enamel organs of the teeth.


Teeth



Teeth form from a series of epithelial/mesenchymal interactions along the dental lamina. In 27-mm embryos individual dental laminae expand into little ectodermal (dental) sacs surrounded by vascular mesenchyme. The ectoderm proliferates to form an *enamel organ* which surrounds a local portion of neural crest mesenchyme, the *dental papilla*; together this unit is a *tooth bud* or *germ*. The enamel organ initially forms a cap over the dental papilla then later it expands into a

bell shape, the inner layer tightly adherent to the dental papilla and separated from the outer layer by accumulated glycosaminoglycans (GAGs). The inner cells of the enamel organ differentiate into *ameloblasts*, and the underlying mesenchymal cells into *odontoblasts*. Tooth development is further considered on page 111 ; the interactions associated with tooth development are considered below.

Both the deciduous and permanent teeth are formed as above. The permanent teeth develop in accessional positions from the lingual aspects of the existing tooth germ; however, the tooth germs for the 12 permanent molar teeth develop from posterior extensions of the dental laminae on each side of both jaws. Calcification begins in both deciduous and permanent teeth before birth; the deciduous teeth have well-developed crowns by full term, whereas the permanent teeth remain as tooth buds.

Just as the neural crest mesenchyme is responsible for the patterning of the pharyngeal arches, so it directs the pattern of tooth development. Thus, dental papilla mesenchyme is able to induce the formation of teeth in non-oral epithelium, and can specify the type of tooth produced, i.e. incisor or molar. Reciprocal interaction between the cells of the tooth germ in response to the extracellular matrix they secrete occurs, i.e. secretion of predentin by odontoblasts stimulates the differentiation of the inner enamel organ into ameloblasts which secrete enamel.

When cranial neural crest is cultured alone it will differentiate into cartilage. If it is recombined with limb epithelium then cartilage and bone will form. However, when cranial neural crest is recombined with mandibular epithelium, salivary islands, hair and teeth form as well as cartilage and bone. Thus the mandibular epithelium is essential and specific for the development of teeth. At a local level, early (9–11.5 days) recombination of mouse mandibular epithelium and second arch mesenchyme results in teeth in 90% of cases, whereas the reverse recombination, second arch epithelium and mandibular arch mesenchyme, does not produce teeth. Later recombination experiments (11.5–12 days), where first arch mesenchyme is grafted with second arch epithelium, will produce teeth, leading to the conclusion that the crest mesenchyme becomes specified to produce teeth after day 12 (Kollar & Mina 1991 ). This specification can be changed experimentally. If presumptive incisor region of the mandibular epithelium is recombined with predetermined molar papillae from post-day 12 tooth germs, the shape of the teeth can be redefined by the epithelium and incisiform teeth develop.

The mesenchymal dental papilla can influence epithelia from different species; thus recombination of dental mesenchyme from 16–18-day mouse with oral epithelium from the mandibular epithelium of the chick resulted in tooth formation (Kollar & Fisher 1980 ). This is the more surprising as chicks do not normally develop teeth. Similarly recombination of chick arch mesenchyme and 10-day mandibular epithelium from the mouse resulted in tooth formation (Kollar & Mina 1991 ). In the latter case the epithelium initiated the dental papilla development.

Anomalies of Facial Development

Congenital malformations consequent upon arrest of development and failure of fusion of

components in the formation of the face and palate are not uncommon. At the simplest, one maxillary prominence may fail completely to fuse with the corresponding premaxillary region (globular prominence), leading to a persistent fissure between the philtrum and lateral part of the upper lip on that side, *cleft lip* (less appropriately 'hare' lip). A similar but rare malformation follows failure of fusion between the maxillary prominence and the lateral nasal prominence, facial cleft, in which the nasolacrimal duct persists as an open furrow, a condition usually associated with cleft lip on the same side. The palatine processes may fail to fuse with each other and the nasal septum to variable degrees. In its severest form fusion is wholly lacking, leaving a wide fissure between the palatine processes through which the nasal septum is visible. On each side the premaxillary parts of the palate are separated from the maxillary palatine processes by clefts which are continuous ventrally with bilateral clefts in the upper lip. In such cases the philtrum is a separate entity, continuous cranially and dorsally with the nasal septum. The floor of the nasal cavity is deficient throughout its extent and the choanae are not completed. Many varieties of milder degrees of cleft palate have been observed; the commonest type is unilateral, only one side of the nasal cavity being in communication with the mouth and the extent of the cleft being variable. In the mildest forms only the soft palate is cleft, or even merely the uvula. Such examples of arrested development may be associated with disturbances in embryonic nutrition during the second and the third months of gestation and the grosser varieties are usually coupled with malformations in other regions of the body (p. 333). In such cases the premaxillary region protrudes, with associated extension forwards of the nasal septum. For discussion see Latham (1973). Certain midline anomalies are rarely encountered, i.e. *median cleft lip* (true hare lip), *cleft nose* and *cleft lower jaw*. More common are minor degrees of *cleft chin* and *micrognathia*—underdevelopment of the lower jaw.


The further growth of the face during the fetal period has received little attention, although this period is by no means characterized entirely by incremental growth. It is during fetal life that human facial proportions develop (p. 371, Fig. 4.28). The facial and cranial parts display different patterns of growth, though each influences the other. For an interesting analysis of the data observed from 280 fetuses consult Lavelle (1974).

Caudal Pharyngeal Arches



Second Pharyngeal Arch


The ectoderm covering the outer aspect of the second pharyngeal arch originates from a strip of ectoderm lateral to the metencephalic neural fold (3.145), as does the otic placode (these placodal cells are located more laterally than the trigeminal placode in the 3-somite chick embryo). The cartilaginous element of the second arch (Reichert's cartilage) extends from the otic capsule to the midline on each side. Its dorsal end separates and becomes enclosed in the developing tympanic cavity as the *stapes*. Thereafter the cartilage gives rise to the *styloid process*, *stylohyoid ligament*, the *lesser cornu* and probably the *cranial rim* of the body of the *hyoid bone* (3.143).




The muscles of the second arch derive from somitomeres 4 and 5. For the most part the muscle

mass migrates widely but retains its original nerve supply from the facial. The *stapedius*, *stylohyoid* and *posterior belly of digastric* remain attached to the hyoid skeleton, but the *facial musculature*, *platysma*, *auricular muscles* and *epicranius* all lose connection with it (3.144 ). Their migration is facilitated by the early obliteration of some of the first groove (cleft) and pouch (see below). (This cleft, the spiracle in fishes, is already much reduced in all but the earliest vertebrates.)


Third to Sixth Pharyngeal Arches

The ectoderm adjacent to the myelencephalic neural fold, down to the level of somite 3, develops to cover the third and fourth pharyngeal arches, a much smaller distribution than that of the more rostral arches. The ectoderm in this region also gives rise to placodal cells which contribute to the petrosal and nodose ganglia. Chondrification does not occur in the dorsal parts of the skeletal elements of the third to sixth arches. The ventral cartilage of the third arch becomes the *greater cornu* of the *hyoid bone* and the *caudal part* of its *body*. (The whole of the body may be formed from the third arch cartilage.) Alternatively, the hyoid body may be derived from cartilage formed in the base of the hypobranchial eminence (p. 175 ) and thus from third arch tissue alone (Frazer 1926 ) , acquiring its connection with the second arch cartilage secondarily.

The final adaptations of the cartilages of the skeletal elements in the fourth, fifth and sixth arches are a source of disagreement, but the following represents a fairly general view. The *thyroid cartilage* develops from the fourth and fifth arches, which may also give rise to the *arytenoid*, *corniculate* and *cuneiform cartilages*. The *cricoid cartilage* may be derived from the sixth arch cartilage, or it may be a modified tracheal ring. The *epiglottis* is developed in the substance of the hypobranchial eminence and probably not from 'true' branchial cartilage (3.144 ).

The paraxial mesenchyme from somitomeres 6 and 7 migrates to the third arch and somitomere 7 alone appears to invade the fourth arch (Trainor et al 1994 ). Somitomeric muscle was not identified in the sixth arch in the mouse. The muscle masses are adapted to form the musculature of the *pharynx*, *larynx* and *soft palate*. The *stylopharyngeus* can be attributed to the third and the *cricothyroid* to the fourth arch (3.144 ). The rest of the laryngeal muscles are derived from the sixth arch and used extensively for vocalization; thus they may not be represented to the same extent in non-human species. The precise origin of the remaining palatal muscles and the pharyngeal constrictors is uncertain in man. A mixed origin, partly from paraxial mesenchyme and partly from adjacent myotomes, has been attributed to sternocleidomastoideus and trapezius (McKenzie 1955 ).

Nerves of the Pharyngeal Arches


The nerves of the pharyngeal arches immediately enter the dorsal ends of them (3.139 ). They are typically mixed, their motor component supplying the muscles of the arch and their sensory fibres innervating the skin and mucous membrane derived from the region. In fish the trunks of the nerves and their ganglia are close to the dorsal ends of the true clefts existing in these forms, each sending a post-trematic branch into its own arch and a pretrematic branch into the arch





cranial to this. In mammals, some have claimed that both types of branch can be identified in the first arch, but only a single nerve can be identified with certainty in the second to sixth arches, with the exception of the fifth, the nerve of which is unknown and may have disappeared.

The trigeminal mandibular division is the post-trematic nerve of the first arch; the chorda tympani, or greater petrosal, has sometimes been regarded as its pretrematic nerve derived from the facial. The latter supplies the second arch, the glossopharyngeal the third, the superior laryngeal branch of the vagus the fourth and the latter's recurrent laryngeal branch the sixth. In lower vertebrates the fifth arch is also supplied by a vagal branch. Other branches that have, on occasion, been proposed as pretrematic are the tympanic branch of the glossopharyngeal and the auricular branch of the vagus. However, none of the foregoing fulfil sufficient criteria for them to be classified as pretrematic with confidence.

The difference in the courses of the recurrent laryngeal nerves can be explained by the development of the aortic arch arteries. In arches 1–5 the arch nerve enters rostral to its aortic arch artery. However, the nerve enters its sixth arch **caudal** to the aortic arch artery, retaining this position on the left side and hence being caudal to and looping round the ligamentum arteriosum in its final disposition. However, on the right, owing to the disappearance of the dorsal part of the sixth aortic arch artery and the whole of the fifth, the nerve loops round the caudal aspect of the **fourth** aortic arch artery, i.e. the subclavian artery.

Muscle of the Pharyngeal Arches

The muscles of the face and neck (**3.144** ), sometimes described as branchiomic because of their origin within the pharyngeal (branchial) arches, develop from a rostral continuation of the paraxial mesenchyme which, in the trunk, segments to form somites. Within the trunk somites give rise to medial skeletal elements: sclerotomes, which combine to form the vertebrae, lateral myotomic populations; myotomes, which form all of the striated muscle of the trunk and limbs; and limited dorsolateral connective tissue populations which contribute to the dermis over the dorsal surface dermatomes. Experimental quail–chick chimeras have permitted examination of the paraxial mesenchyme in the head to see if similar tripartite fates are available.

Although the paraxial mesenchyme in the head is unsegmented, a segmental pattern was described by Meier (1979 ) who noted seven *somitomeres* each side of the rostral notochord and beneath the overlying neural plate (see p. 154 ). Portions of paraxial mesenchyme, medial and lateral to the folding neural plate, were transplanted from quail embryos to chick and the fate of the cell populations followed (Couly et al 1992 ). At the 3-somite stage the cell density is much higher in the lateral paraxial mesenchyme than in the medial. Apart from the rostral regions of the medial paraxial mesenchyme, which in the avian embryo appeared to contribute to the ocular muscles (these in the main originate from prechordal mesenchyme; see p. 274 ), the medial mesenchyme gave rise to limited skeletal structures, for example part of the sphenoid and otic capsule, and connective tissues, including the mesencephalic and metencephalic meninges, but no muscles. In contrast, the lateral paraxial mesenchyme developed into the muscular lineages of the pharyngeal arches.

It is interesting that the fate of medial and lateral paraxial mesenchyme corresponds to the medial and lateral fates of the somites. The limited contribution to the dermis seen in the somites has no equivalence in the paraxial mesenchyme. In the head the dermis is formed by neural crest mesenchyme which also has the ability to develop calcified structures.

Prior to the formation of any skeletal elements in the arches, myoblasts migrate from the paraxial mesenchyme to the sites where overt muscle differentiation will occur and form premuscle condensations. The pattern of primary myotube alignment for any one muscle is specified by the surrounding neural crest mesenchyme and is not related to the source of the myoblasts. The rate and pattern of muscle maturation are closely associated with the development of the skeletal elements, such that muscles may attain attachments to one skeletal element but remain without additional attachments until the appropriate elements develop (McClearn & Noden 1988^[1]). Figure 3.148^[2] shows the relationship between the somitomeres and the muscle masses migrating to each arch.

Pharyngeal Grooves

Modification of the external contours of the arches occurs as the skeletal and muscular elements develop. The modification of the external *pharyngeal grooves* or (less appropriately) *clefts* produces the smooth contour of the neck. The concurrent development of the internal *pharyngeal pouches* also contributes to this process.







The first pharyngeal groove is obliterated ventrally, as in all but the most ancient vertebrates. In man its dorsal end deepens to form the epithelium of the external acoustic meatus and the external surface of the tympanic membrane. (For details see p. 262^[3].)

At the dorsal ends of the first, second and fourth pharyngeal grooves thickened patches of ectoderm appear, the *epibranchial placodes*. These are closely related to the developing ganglia of the facial, glossopharyngeal and vagus nerves, to which they contribute (p. 224^[4]): these, and other placodal cells (*dorsolateral* and *suprabranchial*) also contribute to the trigeminal and vestibulocochlear ganglia (see 3.103^[5]).


At the end of the fifth week the third and fourth arches are sunk in a retrohyoid depression, the *cervical sinus*. Cranially the sinus is bounded by the hyoid arch, dorsally by a ridge produced by ventral extensions from the occipital myotomes and by mesenchyme developing into sternocleidomastoid and trapezius. Caudally, the smaller *epipericardial ridge* separates the sinus from the pericardium and curves cranially near the midline and then with its fellow reaches the lingual swelling of the mandibular prominence and the hypobranchial eminence. Myoblasts from the occipital myotomes migrating to the tongue follow the epipericardial ridge together with the hypoglossal nerve. The long held view that the cervical sinus is obliterated by caudal growth of the hyoid arches to fuse with the cardiac elevation, excluding the succeeding arches from any part in the formation of the skin of the neck, has been criticized; an alternative view is that the sinus is reduced by gradual approximation of its walls from within outwards. It should be noted,


however, that some contend that the surface course of the second groove persists as the curved submandibular *cervical flexure line*. Whatever the mechanism a smooth epidermal covering to the neck results with platysma (a second arch muscle), bounded both superficially and deep by superficial fascia, passing along the neck to the anterior thoracic wall.

Pharyngeal Pouches

The first four pharyngeal pouches appear in sequence craniocaudally, and their endoderm approaches the ectoderm of the overlying pharyngeal grooves to form thin *closing membranes* (3.139 , 140 ). The blind recesses of the second, third and fourth pouches are prolonged dorsally and ventrally as angular, wing-like diverticula. From the fourth a diverticulum grows caudoventrally and is at first demarcated from the pouch by a groove in which may occur a transient fifth aortic arch artery. From this diverticulum a fifth pouch may develop and establish a connection with the ectoderm. The remainder of this diverticulum is the *ultimobranchial body*. This, together with the fourth pouch and the transitory fifth, when present, constitute the *caudal pharyngeal complex*. Its communication with the cavity of the pharynx is the *common pharyngobranchial duct*. The ultimobranchial body is almost a constant feature of vertebrate development (Watzka 1955 ). Its form in the human embryo, however, has been a matter of controversy. Apparently it is incorporated into the rest of the caudal pharyngeal complex and contributes to the development of the lateral thyroid rudiment (p. 177 ). Ultimobranchial bodies exist in the adults of many lower vertebrates and *calcitonin* has been isolated from such tissue (Copp et al 1967 ). There is thus a strong presumption that the parafollicular cells of the human thyroid gland, which are a source of calcitonin, are derived from ultimobranchial tissue. The further development of the endodermal derivatives of the pharyngeal pouches is intimately associated with that of the mouth, pharynx and larynx, and is considered with them (p. 176 ).

Rhombomeres, Hox Genes and Arch Development

It has been seen in the above accounts that the cranial neural crest proliferates to form a significant mesenchymal population in the head, face and pharyngeal arches which controls the pattern of development of the face and arches, specifying the position of muscles, nerves and blood vessels. Experimental studies have shown that if first arch (mandibular) crest is grafted into the hyoid (second) arch, mandibular structures form, suggesting that the differentiation pattern of the second arch paraxial mesenchyme and surface ectoderm was redefined by the new crest mesenchyme (Noden 1988 ). Other experiments on tooth development (see above) illustrate the same phenomenon. These experiments, however, do not suggest whether the crest cells gain their patterning ability before leaving the neural plate or afterwards during their migration to the arches.

The neural crest cells migrate from the neural folds of the diencephalon, mesencephalon, metencephalon and myelencephalon; crest cells do not arise from the prosencephalic neural folds. At the time of crest migration the hindbrain (rhombencephalon) is composed of a repeating pattern of bulges, the rhombomeres (see p. 241 ), segmental units seen in the brains of all developing vertebrates. Single-cell marking experiments show that rhombomeres operate as distinct compartments with lineage restriction. There are eight rhombomeres identified in the

hindbrain. Labelling of crest cells along the neural folds prior to migration has revealed a relationship between the sites of emergence of the crest cells and the rhombomeric epithelium (Lumsden et al 1991^[1]). Neural crest cells originate from three discontinuous levels and migrate ventrally in three distinct streams (3.106A^[2]). Crest cells from rhombomeres 1 and 2 contribute to the trigeminal ganglion and produce first arch mesenchyme, crest cells from rhombomere 4 contribute to the facial and vestibulo-acoustic ganglion and produce second arch mesenchyme, while crest cells from rhombomere 6 contribute to the superior petrosal ganglion and produce third arch mesenchyme. Two axial levels, rhombomeres 3 and 5, do not contribute to the emergent neural crest. However, crest cells migrating from rhombomeres 3 and 5 have been isolated in vitro, suggesting that in vivo the even-numbered rhombomeres may exert a dominant negative effect upon the odd numbered, suppressing neural crest production (Graham et al 1993^[3]). Such suppression, by segregating the crest into three distinct streams, may ensure the specific filling of each of the pharyngeal arches and the correct development of each of the individual cranial ganglia. The specification of the neural crest thus occurs before it migrates from the neural folds.

The axial homeobox genes, *Hox-a* and *Hox-b* (see p. 228^[4]), are expressed in the rhombomeres and in neural crest cells from the point of origin, during migration and after migration has ceased. Each pharyngeal arch expresses a different combination of *Hox* genes in a segment restricted manner (Hunt et al 1991^[5]; 3.106^[6], 148^[7]). The exact relationship between *Hox* expression in the rhombomeres and later in the arches is not yet clear. For example, *Hox-b1* is delineated sharply in rhombomere 4 and later in arch 2; however, *Hox-b2* is expressed in all rhombomeres caudal from rhombomere 3, yet rhombomeres 3 and 5 do not produce migratory crest cells. Figure 3.148^[8] shows the extent of *Hox* expression in the rhombomeres, the neural crest and the surface ectoderm.

Disruptions of the *Hox* genes cause failure of normal crest cell proliferation and migration, producing anomalies similar to human congenital disorders, for example DiGeorge's syndrome (see p. 228^[9]).

Head Development and Evolution

The developmental mechanisms which operate within the trunk are different from those operating in the head: an observation which could be used to deduce that the head evolved by a different route from the other axial structures, using different cell populations which do not respond to, or differentiate earlier than, the inducers in the trunk region.

The vertebrate head is especially different from the 'cranial end' of its nearest relations, the cephalocaudates. They have, in common with vertebrates, segmented muscle blocks, a dorsal hollow nerve cord, gill slits and a notochord. However, they have no clear head, no obvious tripartite brain, no neural crest or ectodermal placodes, no paired sense organs or cranial ganglia. Amphioxus and other cephalocaudates may be considered to be distant relations of vertebrates; however, no link can be postulated that would demonstrate the gradual evolution of

cephalization.

Until now, we have had few tools with which to examine the complexity and **comparative** nature of head development in extant species of vertebrates **and** cephalocaudates. The data now being generated by molecular biological studies on head development will have far-reaching effects and take much time to interpret.

A hypothesis of head evolution which is suggested by examination of the development of the head has been put forward by Gans and Northcutt (1983^[1]) and Northcutt and Gans (1983^[2]). They propose that the rostral part of the head, including the sense organs, prosencephalon, mesencephalon and surrounding skull, is derived from the neuroectoderm. Experimental studies have confirmed that the 'prechordal' skull, i.e. that part rostral to the notochord (Couly et al 1992^[3]), which surrounds the expanded rostral brain, is formed from neural crest, a population of neuroectoderm which invaginates between the neural tube and epidermal ectoderm, after neurulation. The neuroectoderm also gives rise to the sense organs via a series of ectodermal placodes, regions of neuroectoderm which do not separate from the epidermal ectoderm until the invaginated neural crest migrates beneath them. Between them, the cell populations produced by the neural crest and ectodermal placodes produce all of the sensory ganglia and sense organs within the head (the eyes, which are derived directly from the neural tube, are excluded from this group).

The neural crest also provides a new mesenchymal population which fulfils a role similar to that of the somatic and somatopleuric mesenchymes within the trunk. Specific interaction between the sclerotomal portion of the somite and the perinotochordal matrix promotes chondrogenesis around the notochord and neural tube resulting in the formation of the vertebrae. Condensations of somatopleuric mesenchyme within the limb have a chondrogenic fate when the cell density is high, and somatopleuric mesenchyme can be induced to follow this lineage in culture if the cells are arrested from migration and kept at high density. Neural crest cells, which never follow a chondrogenic pathway in the trunk, are able to differentiate into chondrocytes and other connective tissues in the head, and they are able to pattern the development of the facial primordia in the same manner as somatopleuric mesenchyme can pattern the limb. The mechanisms by which this occurs is not clear although the 'flypaper model', specifying the pattern of cranial chondrogenesis (Thorogood 1988^[4]), suggests that the neural crest responds to similar cues and in a similar manner as the sclerotomes (see p. 274^[5]).

It should be noted that the vertebrate head is formed not only by addition of neural crest in the rostral region but also by incorporation, in the caudal region, of an increasing number of vertebrae. The Agnatha have no vertebral contribution to the skull; the amphibians and selachians incorporate three occipital somites, whereas in the vertebrate skull all five occipital somites are included. This caudal enlargement contributes to the general increase in the volume of the skull around the expanding rhombencephalon at the same time as the crest-derived rostral portions of the skull surround the prosencephalon.

Mapping of the neural plate in the chick has shown that the prechordal skull is formed rostral to

the adenohypophyseal placode from ectodermal and neural crest cells located in the neural folds. From rostral to caudal the neural folds produce the adenohypophysis (in the midline) and then on each side the olfactory ectodermal placode, the frontonasal ectoderm, the calvarial ectoderm and the cephalic neural crest (3.145). The ectoderm of the first pharyngeal arch is found lateral to the cephalic neural crest and it migrates rostrally and medially to contribute to the face (3.145). There is a neural crestfree gap over rhombomere 3 (see below) that separates presumptive frontonasal/ maxillary/mandibular cells from the second arch crest. Few or no crest cells are formed by neural folds at the level of the otic placode (rhombomere 5).

After neurulation, many structures present in the head of extant vertebrates are segmentally organized. For example, in the rhombencephalon there are ridges which divide the hindbrain into rhombomeres (3.148); subjacent paraxial mesenchyme is arranged as definitely segmented occipital somites and, more rostrally, possibly segmented somitomeres. More lateral and ventral locations contain the ectodermal placodes, the embryonic aortic arch arteries and the pharyngeal pouches. However, this segmentation is seen only caudal to the hypophysis, each side of the notochord. Thus the transient segmental nature of cephalic development is taking place in the 'ancestral' or 'old' head and brain. However, this paradigm is only partially supported by studies examining the expression of *Hox* genes in the developing brain.



Hox genes are expressed along the embryonic axis in invertebrate and vertebrate embryos. Recent cloning of *Hox* genes from cephalochordate embryos has shown an amphioxus *Hox* gene *AmphiHox-3* (Holland et al 1992) homologous to the mouse *Hox-2.7* gene. The rostral limit of expression of this gene in amphioxus is at the level of the 4/5 somite boundary at the neurula stage and at later stages within a spatially restricted domain of the developing nerve cord. Homologous gene expression in vertebrate embryos corresponds to the rhombomere 4/5 boundary. Holland et al propose that the structures expressing these genes in the two body plans are homologous. They suggest that this evidence supports the hypothesis that the vertebrate head evolved by elaboration and expansion of a pre-existing cranial region rather than by production of a new rostral portion. The utilization of extensive neural crest populations in the head may have resulted because it was a source of mesenchyme which could be modified and adapted without simultaneous reorganization of the trunk; the developmental flexibility of the crest population could promote an evolutionary flexibility and produce the diversity seen in vertebrate species today.


Noden (1991) adds a note of caution to the general interpretation of the developmental processes within the head, especially with the eruption of molecular biological applications for studying embryonic development. He recognizes that our present understanding of the morphology of development, of the patterns of cell movement, commitment and interactions which lead to the spatial assembly of complex arrays, is as yet inadequate to provide a basis for interpreting molecular analysis. The resolution of these challenges in the understanding of head development is awaited with interest.

Appendicular Skeleton and Muscles

The appendicular skeleton and muscles arise from both paraxial mesenchyme (the epithelial somite) and lateral plate mesenchyme (somatopleuric).

Morphological Changes in the Limbs

The limbs develop via a continual series of complex epithelial/ mesenchymal interactions initiated in the lateral body walls. The proliferating somatopleuric mesenchyme forms a ridge externally, ventrolateral to the somites, which extends caudally from the most caudal (sixth) pharyngeal arch, finally tapering towards the tail. Interaction of *specialized regions of the somatopleuric mesenchyme* with the overlying ectoderm gives rise to local, thickened regions of surface ectoderm and proliferation of the underlying mesenchyme; this specifies the position of the future limb buds. At the site of each putative limb the ectoderm forms a longitudinal ridge of high columnar epithelial cells, the *apical ectodermal ridge (AER)* (3.149 , 150 ). The AER and the underlying, specialized somatopleuric mesenchyme are termed the *progress zone*; this remains at the distal tip of the limb until the digits are formed. The progress zone controls the orientation and progression of limb development and specifies the position of the skeletal elements. The somatopleuric mesenchyme controls the specific developmental fate of the overlying ectoderm and within the limb becomes the skeletal and connective tissue elements. Precursor muscle cells and neurons migrate into the limb somewhat later. Consistent with the craniocaudal progression of development, the upper limb develops in advance of the lower. The earliest signs of limb development are seen in stage 12 (26 days) embryos. A ridge is visible along the lateral longitudinal axis of the body wall opposite somites 8–10, at the level of the entrance to the cranial intestinal portal; this is the upper limb bud. By stage 13 the lower limb bud is also visible.

The upper limb bud enlarges, protruding laterally from its elliptical base at the body wall as a flattened plate, with a curved border and an AER forming its distal tip; it also has initially equal and relatively flat *dorsal* and *ventral ectodermal surfaces*, and a somatopleuric mesenchymal core. For descriptive, experimental and conceptual purposes it has been necessary to define and name various 'axes', borders, surfaces and lines in relation to the bud (3.150 ). (However, some minor variation in *terminology* will be noted when human development is compared with basic tetrapod—amphibian, reptilian and also avian—development. Mechanisms, nevertheless, remain similar.) An imaginary line from the centre of the elliptical base of the bud, through the centre of its mesenchymal core, to the centre of the apical ectodermal ridge, defines the *proximodistal axis* of the bud (for long, in descriptive embryology, known simply as **the axis**). Named in relation to the latter, the limb border cranially placed is the *preaxial border* and that caudally placed is the *postaxial border*. (In tetrapods and birds, the latter are termed anterior and posterior borders, respectively; see below.) Any line passing through the limb bud from preaxial to postaxial border, (and orthogonal to the proximodistal axis) thus constitutes a *cranio-caudal axis*. The dorsal and ventral ectodermal surfaces thus clothe their respective aspects from preaxial to postaxial borders. Thus, any line passing from dorsal to ventral aspect (and orthogonal to both proximodistal and craniocaudal axes) constitutes a *dorsoventral axis*. (It should be noted here that the terms *dorsal* and *ventral axial lines* are to be used exclusively in relation to developing and definitive patterns of cutaneous innervation of the limbs and their

associated levels of the trunk.

Early differential growth of parts of the limb bud result in two main changes to the originally symmetric axes of the limb:

- (1) The dorsal aspect of the limb grows faster than the ventral; this causes the limb bud to curve around the body wall; the ventral surface of the limb which is closest to the body wall remains relatively flat but the dorsal surface bulges into the amniotic cavity; the originally laterally facing AER becomes increasingly directed ventrally.
- (2) Slightly later the preaxial border grows faster than the postaxial, resulting in a further shift of the AER caudally rather than ventrally. These reorientations in the upper limb form the shoulder, arm and forearm; however, their effects cannot be seen until later (see below).

By stage 13 (28 days) the upper limb bud is curving ventrally while the lower limb bud is still directed laterally; in stage 14 embryos the preaxial border has started to lengthen in the upper limb but not yet in the lower. The upper limb at this stage is opposite the developing ventricles of the heart; the lower limb is closely associated with the wide umbilical cord. In stage 15 the upper limb can be subdivided into definite regions. The proximal portion of the limb still shows the dorsal bulge and ventral curve—this is the shoulder region and upper arm region; the next distal portion which was derived from the increase in the length of the preaxial border can now be identified as the forearm. The most distal portion is now expanded into a flattened hand plate.



At stage 16 the limbs appear much more substantial. The upper limb is sometimes close to the body wall and sometimes abducted; the lower limbs do not curve close to the body wall as the umbilical cord is very wide at this time. The hand plate has the first indications of digit rays and the lower limb has an early foot plate.

By stage 17 the upper limb has an elbow region and digit rays; in advanced members of this group the hand plate has a crenated rim indicating the beginning of tissue removal between the digits. The lower limb still has a flattened foot plate. Although a hip region can be seen there is no true knee as yet.

In stage 18 (44 days) embryos the foot plate has digit rays and there is further crenation of the hand plate between the digit rays. The lower limb appears to be flexed at the hip and abducted with the knee bent; this gives the appearance that the knee is facing laterally. There is very little skin of the thigh visible; the soles of the feet face the umbilical cord.




Changes during stages 19–23 are concerned with growth of the limbs and separation of the digits. The hands are now curving over the cardiac region. The distal phalangeal portions of the fingers enlarge at stage 21 forming the nail beds. This can be seen on the separated toes at stage 23. The feet can finally touch at stage 21 when the umbilical cord becomes proportionally smaller and the embryo larger.

Concepts of Limb Development




Limb development may be conceptualized as resulting from a series of ectodermal/mesenchymal interactions (3.150 , 151 ). Such concepts are supported by experimental evidence from amphibian, avian and reptilian species which demonstrate a remarkable conservation of developmental processes. Chimeric experimentation has further revealed the specific fates of cell populations within the developing limb. The demonstration of conserved homeobox-containing genes in the developing limb (see below) may however require some reinterpretation of these concepts to reconcile the molecular model with the traditional model.

Progress Zone (AER-Mes)

The outgrowth of a limb bud is controlled by the apical ectodermal ridge (AER) and the *underlying somatopleuric mesenchyme*. The epithelium seems to control the developmental stage of the limb and the somatopleuric mesenchyme controls the type of limb, interpreting the temporal information from the AER in a proximodistal developmental progression. These two tissue arrangements form the *progress zone*, a region which is believed to be the site where assignments are made to cell populations in the limb. As cells leave the progress zone, their *proximal/distal value* becomes fixed. Once the mesenchyme has been assigned it specifies the developmental pattern of the overlying ectoderm.

The work of Zwillling (1972 , Saunders et al (1976 , Hinchcliffe and Johnson (1980 ) has provided much evidence of limb morphogenesis. The knowledge may be summarized as follows:


- The AER and underlying mesenchyme provide the orientating influence for limb outgrowth. Removal of the AER results in cessation of limb development; insertion of a second AER results in two axes of development: there is duplication of distal structures from the graft onwards.
- Replacement of the underlying mesenchyme with any other mesenchyme results in no limb development: only 'limb' mesenchyme will promote limb bud formation; however, replacement of upper limb mesenchyme with lower limb mesenchyme does support limb growth but leads to the development of leg structures. In addition the leg mesenchyme will pass information back to the local ectoderm causing appropriate leg feather (in chick) development. It is reasoned that the mesenchyme beneath the AER provides an 'AER maintenance factor' which is essential to the function of the ridge.


The *temporal* nature of the information passing from the AER to the underlying mesenchyme was illustrated in a series of experiments by Summerbell (1974 ). A graft of a young limb bud to an older one with the progress zone removed results in duplication of limb elements. Conversely a graft of an old progress zone onto the stump of a younger limb produces a limb with intermediate sections missing (see 3.150 ). The progress zone behaves independently as if no communication concerning positional values travels in a proximodistal direction. As cells leave the progress zone their proximodistal values are specified (3.150 .

In grafting experiments only whole limb bones develop. Eight states of the progress zone can be described: i.e. humerus, ulna-radius, carpals I, carpals II, metacarpals, phalanges I, phalanges II, phalanges III, each state taking approximately 8 hours. Summerbell and Lewis (1975^[4]) noted that the progress zone behaves like a clock whose ticks are cell-division cycles.


The precision with which skeletal growth occurs is often not appreciated. In calculating the growth in left limbs versus right limbs Summerbell et al (1973^[4]) concluded that the length of the left ulna of a limb did not vary by more than 5% of the length of the right.

Axes of the Limb

The three developmental axes can be identified in the developing limb bud by stage 13 (3.150 ). These are, as noted above, the *proximodistal*, the *dorsoventral* and the *craniocaudal* axes. Each of the three principal axes seem to be specified by different mechanisms. The *proximodistal axis*, as mentioned previously, is controlled by the *progress zone* (i.e. the AER and subjacent somatopleuric mesenchyme). The *craniocaudal axis* is controlled by a small population of mesenchymal cells on the postaxial border of the limb bud, some distance from the AER; this mesenchyme is termed the *zone of polarizing activity* (ZPA). The ZPA specifies digit five; further away from the ZPA digits four, three, two and one develop.

If the ZPA is grafted beneath an AER, duplication of the limb occurs from that time onwards. If the ZPA is grafted onto the preaxial border of the limb a duplicated distal portion grows with the orientation reversed (3.150 ). The *dorsoventral axis* of the limb appears to be controlled by the *ectoderm* of the limb. If the mesenchyme of a limb is removed and dissociated then repacked into the ectodermal sleeve, a limb will develop which has no anterior–posterior axis, i.e. the ZPA has been dispersed. However, the limb does have dorsal and ventral surfaces identified by the directions of the joints and position and type of hair.

Early Skeletal Elements of the Limb

Formation of the cartilage elements of the limb has been suggested to be related to the shape of the limb and the conditions necessary for chondrogenesis. There is an *antichondrogenic zone* beneath the ectoderm of the limb which prevents chondrogenesis within the dermis and myogenic zones (p. 264 ). Foci of chondrogenesis occur in the **centre** of the limb bud where the cell density is highest, then the production of extracellular matrix by these cells encourages chondrogenic differentiation. In more distal portions of the limb, the limb bud widens forming first two centres of chondrogenesis, then later five centres. The AER is believed to control the width of the digital plate which in turn will reflect this width by the number of digits which develop. Zones on the cranial and caudal, or preaxial and postaxial borders of the limb which show preprogrammed cell death can be identified at the same time as the ZPA. These zones limit the length of the AER.

The experimentation concerning these zones was carried out in chick embryos. It is customary in

anamniote embryology to refer to the craniocaudal axis as anteroposterior (with the human anterior and posterior surfaces being termed ventral and dorsal, respectively). This terminology has been retained for many amniote embryos, especially avian. Thus the special zones found on the pre- and postaxial borders of the limb are referred to, in the literature, as *anterior* and *posterior necrotic zones* (ANZ and PNZ). If the length of the AER becomes reduced then fewer digits will form—*oligodactyly*; if the AER is not reduced and becomes longer then more digits will form—*polydactyly*. This latter condition can permit the development of supernumerary digits on either the pre- or postaxial borders. There are other regions of cell death occurring between the digits which result in digital separation, but these occur later than the ANZ and PNZ. The cells between the digits are removed by macrophages. Note cells in the ANZ, PNZ and between the digits undergo apoptosis.

Most of the bones in the appendicular skeleton derive from somatopleuric mesenchyme. Within the upper limb, however, although the *clavicle* and *coracoid portion of the scapula* arise from somatopleuric mesenchyme, the *body and spine of the scapula* are believed to derive from the somites (Chevallier 1977^[4]). No recent studies yet dispute this finding.

Prechondroblasts are present in the upper limb at stage 13 and condensations of cartilage can be detected at stage 16 when the *humeral anlage* can be recognized. By stage 17, when the *radius* and *ulna* chondrify, the branched tips of the radial, median and ulnar nerves have migrated to the distal hand plate. The *carpal bones* chondrify at stage 18 when the hand plate shows notching of the digital rays. In the lower limb the *femur* and *tibia* have formed in cartilage and the sciatic nerve extends distally to the tibia by stage 18 (44 days).

The first evidence of bone formation is seen at the midpart of the diaphysis of long bones at 8 weeks. Vascular invasion of the cartilage matrix precedes the formation of a periosteal collar which extends proximally and distally until it reaches the future epiphyseal level where a *growth plate* will be established. By 10 weeks columns of chondrocytes can be seen at the epiphyseal level of most bones; however, only the lower end of the femur and upper end of the tibia develop ossification centres prior to birth (see p. 684^[5]). The pelvis forms from two hemipelves which each develop from one cartilaginous focus. Ossification of the pelvis commences with the ilium which undergoes endochondral ossification (similar to long bones) at 9.5 weeks.

Development of Joints

Regions of developing cartilage are easily recognized in the developing limb as they have widely spaced cells surrounded by matrix. Between the developing skeletal elements the somatopleuric mesenchyme is more condensed forming plates of *interzonal mesenchyme* which mark the sites of future joints. Their development varies according to the type of joint formed.

In fibrous joints the interzone is converted into collagen, as the definitive connecting medium between the bones involved. In synchondroses it becomes (growth) cartilage of the modified hyaline type, whereas in symphyses the tissue is predominantly fibrocartilage, but retaining narrow para-osseous laminae of hyaline (growth) cartilage. The interzonal mesenchyme of

developing synovial joints becomes trilaminar, due to the appearance of a more tenuous intermediate zone between two dense strata next to the cartilaginous ends of the skeletal elements of the region. As the skeletal elements chondrify and in part ossify, the dense strata of the interzonal mesenchyme also become cartilaginous and cavitation of the intermediate zone establishes the cavity or discontinuity of the joint. The loose mesenchyme around the cavity forms the synovial membrane and probably also gives rise to all other intra-articular structures, such as tendons, ligaments, discs and menisci. In joints containing discs or menisci and in compound articulations more than one cavity may appear initially, sometimes merging later into a complex single one. As development proceeds thickenings in the fibrous capsule can be recognized as the specializations peculiar to a particular joint. In some, however, such accessions to the fibrous capsule are derived from neighbouring tendons, muscles or cartilaginous elements.

Cavitation of the hip, shoulder and elbow joints has been reported at 7–8 weeks. The sacro-iliac joint can be recognized from 7 weeks, its development being slightly different from other synovial joints in that the development of the ilium is ahead of that of the sacrum. Uthoff (1990 [\[4\]](#)) suggests that the initial stages in the process of cavitation of joints is independent of movements but that a full, true joint cavity can only form in the presence of movements.

Generally the literature suggests that all musculoskeletal elements are in their appropriate positions by 10 weeks. For a review of the literature concerning the chronology of events in human embryonic limbs consult O'Rahilly and Gardner (1975 [\[5\]](#)) and Uthoff (1990 [\[4\]](#)).


Limb Musculature

It is now well established that all limb muscle precursor cells originate from the somites (Jacob et al 1986 [\[6\]](#)). These precursor cells are committed at an early stage and can be identified in the lateral halves of the somites (Selleck & Stern 1991 [\[7\]](#); Ordahl 1993 [\[8\]](#)). After the mesenchymal sclerotome cells have migrated from the epithelial somite the remaining dorsolateral portion is termed the *epithelial plate* of the somite (3.131 [\[9\]](#)). Cells from the cranial edge of this plate form the axial musculature whereas cells from the *ventrolateral edge* of those somites opposite limb buds migrate into the limb anlagen. Initially the cells migrate as single mesenchyme-like cells, then later in groups; they are surrounded by a non-random, structured network of extracellular fibrils. The migrating cells branch at their leading ends into filopodia which are in contact with the extracellular fibrils or with other cells. It is thought that the orientation of the extracellular fibrils may direct the migration of the cells. The precursor muscle cells are, however, not competent to produce limb muscles prior to their migration into the limb, and it is thought that the somito–somatopleural migration is a time when precursor myogenic cells acquire their responsiveness to the somatopleuric connective tissue.

The proliferation of the limb bud is controlled at the distal tip where the somatopleuric mesenchyme and the overlying ectoderm form the AER (p. 291 [\[10\]](#)). The myogenic cells colonize the limb bud in a *proximodistal direction only*, and never reach the most distal portion of the limb where there seems to be a distal boundary for the muscle cells. The speed of migration of myogenic cells into the limb is considered to be constant, since the border of invasion seems to

lag behind as soon as the rate of elongation of the limb bud becomes more pronounced. Myogenic cells are still indifferent regarding their region-specific determination when they first enter the limb. Myogenic cells from a limb will, if grafted into brachial or pelvic somites, assume the myogenic potentialities of the somites and give rise to normal wing or leg musculature. The muscle cells, unlike the somatopleuric mesenchyme, have no 'limbness'. Further, the muscle pattern developed in the limb reflects the pattern of the skeletal elements; duplication or lack of digits is accompanied by the duplication or lack of the corresponding muscles.

Two subpopulations of myogenic cells can be discerned in the limb bud. In the early buds there are mainly *replicating presumptive myoblasts*, considered to be *premitotic*, whereas in later stages there are also *postmitotic myoblasts*. It is interesting that the invading myoblasts are still replicating; this may be a prerequisite for the formation of the considerable amount of skeletal muscular tissue which will develop in the limbs.

The first myogenic cells to arrive in the limb form the principal *dorsal* and *ventral premuscular masses*; it is thought that all classes of tetrapods begin limb muscle development with these blocks which produce all the skeletal muscle in the limb. The blocks of premuscle undergo a spatiotemporal sequence of divisions and subdivisions as the limb lengthens which leads to the individualization of about 19 muscles (3.151 

The axial development of the limb, particularly that controlled by the ZPA, also affects the formation of individual muscles from the premuscular mass, as, if the somatopleuric mesenchyme is dissociated and repacked in an ectodermal sleeve prior to myoblast migration, the muscle masses remain unsplit.

Each anatomical muscle appears as a composite structure; the muscle cells and myosatellite cells are of somitic origin; the connective tissue envelopes and the tendons are of somatopleuric origin. The precise way in which the muscles are anchored to the developing bones by the tendons is not clear.

Embryonic Movements

Embryonic *movements* are vital for development of the musculoskeletal system. As well as effects on the developing muscle they are necessary to align the trabeculae within the bones, the correct attachments of the tendons and the appropriate coiling of the constituent collagen fibres of the tendons. Simple movements of an extremity have been observed sporadically as early as the seventh week of gestation; combined movements of limb, trunk and head commence between

12 and 16 weeks of gestation. Fetal movements related to trunk and lower limb movements are perceived consistently by the mother from about 16 weeks gestation (quickening). Movements of the fetus are often slow, asymmetric twisting and stretching movements of the trunk and limbs, although there may be rapid, repetitive wide-amplitude limb movements. Movements of the embryo and fetus encourage normal skin growth and flexibility as well as the progressive maturation of the musculoskeletal system. It is noted that fetuses with dystrophies which prevent in utero movements develop webs of skin, *pterygia*, passing across the flexor aspects of joints which severely limit movements. A group of congenital disorders, collectively termed *multiple congenital contractures*, may result from genetic causes, limitations of embryonic and fetal joint mobility, or be secondary to muscular, connective tissue, skeletal or neurological abnormalities. These conditions may be recognized on prenatal ultrasound examination by the appearance of fixed, immobile limbs in bizarre positions, or by webbing in limb flexures. Specific syndromes, lethal multiple pterygium syndrome, and congenital muscular dystrophy have been described.

Hox Genes in the Developing Limb

Study of the *Hox* gene clusters in limb development have provided an evolutionary explanation of the tetrapod condition and of the pentate form (Tabin 1992^[4]). Early progathostomes had only a *ventrolateral skin fold* extending along the length of the body axis from which paired fins evolved. Migration of somatopleuric mesenchyme into separate regions of the ventrolateral skin fold specified the position of the early paired appendages. The segments of the body prior to limb development express various *Hox* genes in overlapping preaxial to postaxial domains. The site of limb formation could have a number of overlapping *Hox* gene domains present in the somatopleuric mesenchyme of the lateral body wall; evolution of the limb from this mesenchyme would result in elongation of these domains which then overlap, not like stripes but rather as nested sets, like Russian dolls.

The pelvic girdle is suggested to have developed first with the pectoral girdle reactivating the same genetic programme later, both limbs using *Hox-a* and *Hox-d* genes. There is molecular evidence which suggests that the pectoral girdle may have evolved from a modified branchial arch (Zanger 1981^[4]). The base of the branchial arches expresses *Hox-C-6* which is also expressed in the extreme proximal, anterior region of the forelimb bud, but is not expressed in the hindlimb. This is of interest as chimera studies have shown that the scapula derives from somitic mesenchyme, while the clavicle, coracoid, sternum and pelvic girdles arise from somatopleuric mesenchyme (Gumpel-Pinot 1984^[4]).

Whereas both *Hox-a* and *Hox-d* are present in similar domains in the early limb bud, the *Hox-a* pattern shifts so that *Hox-a* genes show proximal/distal domains and *Hox-d* genes preaxial/postaxial domains. There are five genes in the *Hox-d* cluster which are expressed in the anterior/posterior axis. The nested arrangement of *Hox-d* genes means that the postaxial border of the limb has all *Hox-d* genes (*d-13*, *d-12*, *d-11*, *d-10* and *d-9*) expressed; in the next anterior zone only four genes are expressed (*d-12*, *d-11*, *d-10* and *d-9*), and so on until only *d-9* is expressed. The five genes can specify five different types of digit. Polydactyly can be interpreted as duplication of an existing digit type but not the addition of a new type of digit. The genes do not however directly specify the digit structure, as the same *Hox* genes are expressed in both

fore- and hindlimbs, and in homologous limbs of different species.

Similarities in the Developmental Mechanisms of Facial Primordia and Limb Buds

The proximodistal outgrowth which constitute both the facial primordia and the limb buds are controlled by similar epithelial/mesenchymal interactions and it seems that the local environmental factors which, for example, control the outgrowths of either face or limb will support the other tissue type. Recombination experiments have shown that limb apical ectodermal ridge ectoderm can be maintained by mesenchyme from the three types of facial primordia, i.e. frontonasal, maxillary and mandibular. Of the three types of facial primordia in the chick, frontonasal and maxillary most resemble the limb in that they both contain rods of cartilage and undergo polarized outgrowth. Recombination of frontonasal mesenchyme and younger limb apical ectodermal ridge promoted the development of a cartilage rod in the primordium, forming an outgrowth which resembled an upper beak to the extent that an egg tooth developed. Thus the ectodermal signals from the limb were able to induce facial primordial development.

Reversed experiments, where limb mesenchyme was recombined with facial ectoderm also showed that supportive epithelial/mesenchymal interactions did occur. Interestingly both frontonasal epithelium and mandibular epithelium supported limb mesenchyme without any epithelial thickening, like an apical ectodermal ridge, which would normally be needed for proximodistal development of a limb. However, maxillary epithelium was not able to support limb outgrowth.

These experiments have demonstrated that the developmental signalling is similar but not identical in some facial primordia and the limb bud. One explanation for this could relate to the origin of the facial epithelium. The ectoderm covering the frontonasal process is derived from the neural fold of the prosencephalon (Couly & Le Douarin 1990^[1]), whereas the epithelium of the mandible and maxilla originates from ectoderm lateral to the neural folds (Couly & Le Douarin 1990^[2]). The neural crest mesenchyme within the facial primordia also has different origins, arising from different neural levels (see p. 286^[3]).

The development of an egg tooth provides an epithelial marker for distal differentiation in the frontonasal primordium and suggests that a progress zone operates within the frontonasal mesenchyme, similar to that in the limb. Similar patterns of expression of *MSx1* and *MSx2* are seen in both limb buds and facial primordia. The expression of these genes has been shown to depend on proximodistal position within the limb and this may prove to be the same in the facial primordia.

It will be interesting to see if other ectodermal/mesenchymal primordia such as those which develop around the urogenital membrane and form the external genitalia have similar or different

signalling mechanisms.

Skin and Appendages

Skin is developed from the surface ectoderm and its underlying mesenchyme. *Surface ectoderm* gives rise to the keratinizing general surface epidermis and its appendages, the pilosebaceous units, sudoriferous glands and nail units. It should also be noted that interactions between ectoderm and mesenchyme also give rise to the internal epithelium of the buccal cavity and the teeth (see p. 283📖) and the nasal epithelia (see p. 280📖). The more differentiated descendants of ectodermal cells are known as *keratinocytes* because their most characteristic contents are fibrous proteins called *keratins*, and also to distinguish them from *non-keratinocytes*, immigrant cells of different developmental origin which constitute an important component of the epithelial sheet formed by the keratinocytes, and with which they have a relationship which has been loosely termed 'symbiotic' (see p. 395📖). The non-keratinocytes are: the *melanocytes* derived from the neural crest; the *Langerhans cells* of bone-marrow origin; and *lymphocytes*. The *Merkel cell* is also usually classed as a non-keratinocyte, although it is being increasingly regarded as a modified keratinocyte.


The *dermis*, composed of irregular connective tissue and some of the connective tissue sheaths of peripheral nerves, derives from somatopleuric mesenchyme, for the limbs and trunk, possibly somitic mesenchyme over the epaxial musculature, and from neural crest in the head. Angiogenic mesenchyme gives rise to the blood vessels of the dermis. Nerves and associated Schwann cells, of neural tube and neural crest origin, enter and traverse the dermis to reach their peripheral terminations during development.

Epidermis and Appendages


General (Interfollicular) Epidermis

In the first 4–5 weeks, embryonic skin consists of a single layer of ectodermal cells overlying a mesenchyme containing cells of stellate dendritic appearance interconnected by slender processes, and sparsely distributed in a loosely arranged microfibrillar matrix (3.152👁️). The interface between ectoderm (epidermis) and mesenchyme (dermis), known as the *Basement Membrane Zone* (BMZ), is an important site of mutual interactions upon which the maintenance of the two tissues depends both in prenatal and postnatal life (see below). Ectodermal cells, which characteristically contain glycogen deposits, contact each other at gap and tight junctions. The layer so formed soon develops into a bilaminar epithelium, the *epidermis*, when desmosomes also appear. The basal *germinative layer* gives rise to the definitive postnatal epidermis, and the superficial one to the *periderm*, a transient layer confined to fetal life. The periderm maintains itself and grows by the mitotic activity of its own cells, independent of those of the germinative layer, and expresses different keratin polypeptides. Originally flattened, the periderm cells increase in depth, with the central area containing the nucleus becoming elevated and projecting as a globular elevation towards the amniotic cavity (3.153👁️). The plasma

membrane develops numerous surface microvilli with an extraneous coat of glycosaminoglycans, and cytoplasmic vesicles become prominent deep to it. These developments reach a peak over the period 12–18 weeks, *at which time the periderm is a major source of the amniotic fluid* to which it may contribute glucose; it also has an absorptive function (Lane et al 1987^[1]). From about 20 weeks onwards, the globular protrusions become undermined and pinched off to float free in the amniotic fluid, and the now flattened periderm cells undergo a type of keratinization to form what is regarded as a temporary protective layer for the underlying developing epidermis proper, against an amniotic fluid of changing composition due to the accumulation of products of fetal renal excretion. Up to parturition, periderm squames continue to be cast off into the amniotic fluid, and they contribute to the *vernix caseosa*, a layer of cellular debris which covers the fetal skin at birth.

Proliferation in the germinative layer leads to a stratified appearance with successive layers of intermediate cells between it and the periderm. From an early stage, cells of all layers are packed with glycogen granules (3.154 ) , presumably a source of energy during this early replicative stage of differentiation. Differentiation of these layers is not synchronous throughout all regions of the developing skin, being more advanced cranially than caudally, and on the body progressing from the midaxillary line ventrally. Reduction in glycogen content of the cells is associated with a shift towards biosynthetic activity connected with incipient *keratinization*, manifested by the presence of different enzymes and expression of keratins. Simple, low-weight keratins present from an early date are replaced by those of higher molecular weight associated with differentiation around 10–12 weeks, soon to be followed by profillagrin and fillagrin, and the appearance of keratohyalin granules among filamentous bundles of the uppermost intermediate layer cells at about 20 weeks. The first fully keratinized cells appear shortly afterward. By 24–26 weeks a definite stratum corneum exists in some areas, and by 30 weeks or so, apart from some lingering glycogen in intermediate cells, the interfollicular epidermis is essentially similar to that postnatally (see Breathnach 1971^[2]; Holbrook 1980^[3], for further details).

Melanocytes

Of neural crest origin, these are present in the bilaminar epidermis of cephalic regions as early as 8 weeks (Sagebiel & Odland 1972^[4]). By 12–14 weeks they can reach a density of 2300 per mm² reducing to 800 per mm² just before birth. Keratinocytes regulate the final ratio between themselves and melanocytes via growth factors, cell surface molecules and other signals (Scott & Haake 1991^[5]). Fetal melanocytes produce melanized melanosomes (see p. 389 ) and transfer them to keratinocytes, intrinsic activities clearly independent of ultra violet (u.v.) irradiation, and suggesting functions of melanin other than photoprotection.

Langerhans Cells

These are of bone-marrow origin, are present in the epidermis by 5–6 weeks and are fully differentiated by 12–14 weeks (Breathnach & Wylie 1965^[6]). Their numbers increase at least partially by mitotic division in situ, but at 6 months are only 10–20% of those in the adult. It is

not known if the Langerhans cell functions in immuno-surveillance in fetal skin.

Merkel Cells

These appear in the glabrous epidermis of the palm and sole of the foot between 8 and 12 weeks (Moll et al 1986^[4], 1990^[4]), and later in association with some hairs and with dermal axonal-Schwann-cell complexes. They are now thought to be modified keratinocytes rather than immigrants of neural crest origin (see p. 394^[4]).

Pilosebaceous Unit

Pilosebaceous units develop at about 9 weeks, first in the regions of the eyebrows, lips, and chin, and at progressively later stages elsewhere, proceeding caudally. The first rudiment is a crowding of cells in the basal layer of the epidermis—the *pregerm*. Further proliferation and elongation of the cells leads to a *hair germ*, which protrudes downwards into the mesenchyme where it becomes associated with an aggregation of cells, the primitive *dermal papilla*. With continued downward growth, in a slanted anteroposterior direction, the hair germ becomes a *hair peg*, and when its bulbous lower end envelops the dermal papilla it is known as a *bulbous peg* (3.155^[4]). At this stage three swellings appear on the posterior wall. The uppermost is the rudiment of the *apocrine gland* (present only in some follicles), the middle forms the *sebaceous gland* and the lower one is the *bulb*, to which the *arrector pili muscle* (arising from underlying mesenchyme) later becomes attached. The cells of the lowermost region of the bulb, the *matrix*, divide actively and produce a pointed *hair cone*, which grows upwards to canalize a developing *hair tract*, along which the fully formed hair, derived by further differentiation of cells advancing from matrix, reaches the surface.

Four successive stages of hair follicle development have been noted by Muller et al (1991^[4]). Stage I is characterized by invagination of the epidermis into the dermis which occurs prior to week 11 of gestation; stage II corresponds to the hair germ (see above) and has been described during weeks 13–15 of gestation. The appearance of the putative sebaceous gland from about week 16 is characteristic of stage III, and stage IV is reached when the dermal sheath and the sebaceous glands are differentiated and the hair passes through the skin surface, at about week 18 of gestation.

Sebaceous glands develop independently of hair follicles in the nostrils, eyelids (as tarsal glands) and in the anal region. *Apocrine sweat glands* are formed at the same time as eccrine glands (see below) and are at first distributed widely over the body; however, their number diminishes from 5 months' gestation resulting in the distribution seen in the adult (see p. 406^[4]). For further details of cellular events involved in ontogenetic differentiation of the hair and its sheaths, and of sebaceous and apocrine glands and the hair tract, see Sections 2 and 5. These processes are mirrored in the accelerated and compressed tempo of the differentiation of postnatal skin. Melanocytes are individually present at the hair-peg stage, and abundantly so and quite active in the bulbous peg. Langerhans cells have also been reported (Foster & Holbrook 1989^[4]).

Developing hair follicles are disposed in groups of three. Hairs produced prenatally are called *lanugo hairs*; they are short and downy, lack a medulla, and in certain parts of the body are arranged in a vortex-like manner into tracts. Late in pregnancy, lanugo hairs are replaced by *vellous hairs*, and these in turn by *intermediate hairs*, which are the predominant type until puberty. New follicles do not develop in postnatal skin.

Eccrine Sweat Glands

Eccrine sweat glands are one type of sudoriferous gland. Sweat gland rudiments appear in the second and third months as cell buds associated with the primary epidermal ridges of the finger and toe pads of terminal digits. They elongate into the dermis and by 16 weeks the lower end begins to form the *secretory coil*, within which, by 22 weeks, *secretory* and *myoepithelial* cells are evident. The solid cord of cells connecting the coil to the epidermis becomes the *intradermal duct*, and the lumina of both are formed by dissolution of desmosomal contacts between the cells (Holbrook 1991^[4]). The *intraepidermal duct* is foreshadowed by a coiled column of concentrically arranged inner and outer cells, within which, by fusion of lysosomal vacuoles, a lumen is formed which opens on the surface at 22 weeks (Hashimoto et al 1966^[4]). As with hair follicles, no new eccrine glands develop postnatally. Sweating is said to be possible by 32 weeks, but clearly, has no functional significance in utero.

Mammary Glands

Mammary glands are considered to be much modified sudoriferous glands and as such they are basically ingrowths from the ectoderm, which forms their ducts and alveoli, supported by vascularized connective tissue derived from the mesenchyme. In embryos of about the fifth or sixth week two ventral bands of thickened ectoderm, the *mammary ridges*, extend from axilla to the inguinal region, and in many mammals paired mammary glands develop at intervals along these ridges. In the human embryo the ridges are not prominent features, and only a single pair of glands develops in the pectoral region. The ridges disappear later in embryonic life, but before this the cranial third of each begins to show proliferation to form the two glandular rudiments. Supernumerary rudiments may form anywhere along the path of the mammary ridges and may develop into actual mammae or merely accessory or supernumerary nipples.

As each mammary primordium develops, its ectodermal ingrowth branches into 15–20 solid buds of ectoderm which will become the lactiferous ducts and their associated lobes of alveoli in the fully formed gland. These are surrounded by somatopleuric mesenchyme which forms the connective tissue, fat and vasculature and is invaded by the mammary nerves. By proliferation, elongation and further branching the alveoli are formed and the duct system defined. During the last two months of gestation the ducts become canalized and the epidermis at the point of original development of the gland forms a small *mammary pit*, into which the lactiferous tubules open. Perinatally the nipple is formed by mesenchymal proliferation. Should this fail the ducts open into shallow pits, a malformation known as inverted nipple. At birth the mammary glands are alike in their stage of development in both sexes, and in both some transient secretory activity may be observed, due presumably to circulating prolactin in the mother (Smith 1959^[4]).

In males, thereafter, the mammary glands normally remain undeveloped; in females at puberty, in late pregnancy and during the period of lactation they undergo further, hormone dependent, developmental changes (pp. 418 et seq). For reviews of the prenatal histogenesis and ultrastructural appearances of mammary tissue consult Tobon and Salazar (1974): for postnatal reviews, pages 418 et seq.

Epidermal Ridges

The epidermal ridges are foreshadowed as regularly spaced small down growths of epidermal cells which appear in finger and toe pads during the second and third months. They are known as *primary epidermal ridges*, separated by corresponding dermal ridges, and in the fifth month *secondary ridges* develop, the pattern becomes evident on the surface, and is finalized through further remodelling postnatally (Okajima 1975).

Nails


Fields of proliferative ectoderm appear on the tips of the terminal segments of the digits; they progressively reach a dorsal position, where at about 9 weeks a flattened *nail field* limited by *proximal*, *distal*, and *lateral nail grooves* is apparent. The nail field ultimately forms the *nail bed*, and the primordium of the nail is formed of a wedge of cells which grows diagonally, proximally and deeply into the mesenchyme from the proximal groove towards the underlying terminal phalanx. The deeper cells of this wedge form the primordium of the *matrix* which gives rise to the *nail plate*; this emerges from under a, now proximal, nail fold at about 14 weeks to grow distally over an already keratinized nail bed. The nail matrix is usually considered to have dorsal and ventral (intermediate) components, but there are conflicting opinions as to the extent to which each contributes to the nail, both in ontogeny and postnatally; it is generally agreed that the ventral matrix contributes the major part. It has been claimed that the nail bed additionally contributes up to 20% of the postnatal nail plate (Johnson et al 1991), but embryological studies to date are not clear on this matter. Most texts state that keratohyalin is not involved in the keratinization of nail, but certainly, up to at least 16 weeks, the dorsal matrix granulosa cells which are contributing keratinized cells to the nail plate and *eponychium* (*cuticle*) contain typical keratohyalin granules, and the cells of the ventral matrix next to the nail plate contain single and compound granules similar to those present in granulosa cells of oral epithelia (Breathnach 1971). Similar granules have recently been reported by Picardo et al (1992) in matrix cells of postnatal human toenail.


At 20 weeks, the nail plate entirely covers the nail field (nail bed), now limited distally by a *distal ridge*, which, when the plate projects beyond the tip, becomes the *hyponychium* beneath it. At birth, the histology of the main nail unit components is similar to that postnatally (Zaias 1990); the nail is long and overhanging, and easily falls off during cleansing.

Anomalous development of the epidermis and its derivatives is relatively common. Excessive or diminished growth, or even complete absence, may affect sebaceous or sudoriferous glands and hair, either locally or generally. Similarly, the epidermis may be excessively pigmented




(*melanism*) or lack melanocytes (*albinism*). Excessive keratinization leads to *ichthyosis*. A *naevus* or 'mole' is a locus of excessive pigmentation. Ectodermal dysplasia is a rare condition characterized by fine blond and scanty hair, reduced or absent eyelash and eyebrows. The skin has deficient sweat and sebaceous glands. Teeth are usually peg- or cone-shaped; absence of major salivary glands may occur.

Dermis

The mesenchymal cells underlying the surface ectoderm and early bi- and trilaminar epidermis contact each other by slender processes (3.156 ) to form an intercommunicating network. They secrete a matrix which is rich in ions, water, and macromolecules, proteoglycan/glycosaminoglycans, fibronectin, collagenous proteins of various types and elastin. Further development of these intrinsic components involves the differentiation of individual cell types, fibroblasts, endothelial cells, mast cells, etc., and the assembly of matrix components into organized fibrillar structures—collagen fibres and elastic fibres. During embryogenesis, the matrix is heterogeneous with regard to its biochemical and macromolecular components, both in terms of relative composition, and local and temporal distributions and gradients, so that it is essential to think of matrix differentiation as well as cytodifferentiation during development. Progressive alterations in matrix components underlie many morphological dispositions. The main glycosaminoglycans of embryonic and fetal skin are glycuronic acid and dermatan sulfate. Collagens type I, III, V, and VI are distributed more or less uniformly regardless of fetal age, with some local concentrations of III and V, the levels of which are higher than in postnatal skin. Collagens type IV and VII are predominantly found in the Basement Membrane Zone.

The progressive morphological differentiation of the dermis involves its separation from the subcutis at about the third month; changes in composition and size of collagen fibrils and their organization into bundles amongst which cells become relatively fewer; downgrowth of epidermal appendages; the organization of nervous and vascular plexuses and the relatively late appearance of elastic networks. The papillary and reticular regions are said to be evident as early as 14 weeks, but the overall organization of the dermis continues postnatally (Holbrook 1991 .

Blood Vessels of the Dermis

The dermal vasculature is generally thought to be developed in situ by transformation of angiogenetic mesenchymal cells. Closed endothelial-lined channels containing nucleated red cells are present by 6 weeks underneath the ectoderm (Breathnach 1971 ) and by the eighth week are arranged in a single plane parallel to the epidermis to form ultimately the subpapillary plexus (Johnson & Holbrook 1989 ). A second deeper horizontal plexus is evident by 50–70 days, and both extend by budding as development proceeds. From these plexuses the final patterns of arterioles, venules and capillaries (see p. 399 ) develop, and they are established shortly after birth. Pericytes are also developed from mesenchymal cells.

Lymphatic Vessels

These are formed by mesenchymal cells which become organized to enclose pools of proteinaceous fluid leaking from developing capillaries (Ryan 1991^[4]).

Epithelial/Mesenchymal Interactions in Developing Skin

Epidermal/mesenchymal (dermal) interactions involving mutual inductive mechanisms are important during development and postnatally. They occur at the interface between the two, the *basement membrane zone* (BMZ), the development of which may be considered in morphological, biochemical, and immunological terms.

The basement membrane zone, at the ectodermal stage, consists of the basal plasma membrane of the ectoderm cell, paralleled on the cytoplasmic side by a skein of microfilaments, and beneath it, a layer (0.1–0.2 μm) of microfibrillar-amorphous material deposited by the cell (3.157^[5]). At the bilaminar stage, a definite continuous lamina densa is present, in the assembly of which fibronectin is involved, and it is separated from the basal plasma membrane by a lamina lucida traversed by loosely fibrillar material; similar filaments extend from the lamina densa into the mesenchymal matrix (3.157^[5]).

Hemidesmosomes begin to appear at 8 weeks as stratification starts, and anchoring fibrils at 9–10 weeks. By the end of the third month the basic morphology of the interfollicular BMZ is essentially similar to that postnatally (see p. 397^[6]). Immunocytochemical studies with monoclonal antibodies recognize the temporal onset of BMZ antigenic expression. For example, GB3 antigens (associated with hemidesmosomes) and laminin are shown to be present in the lamina lucida at 6 weeks, and LDA-1 antigen and collagen type IV in the lamina densa at the same time. Antigen LH7:2, associated with anchoring fibrils, is present at 8 weeks. Bullous pemphigoid antigen (hemidesmosomes) and antigens AF-1, AF-2 (anchoring fibrils) and KF 1 are expressed later, and the time of appearance of others is being regularly reported. These observations, combined with morphological ones, are of importance for prenatal diagnosis of genetically-determined diseases such as epidermolysis bullosa (Eady 1994^[7]).

The basal lamina provides a physical supporting substrate and attachment for the developing epidermis, and is thought to be selectively permeable to macromolecules and soluble factors regulating epidermal-dermal morphogenetic interactions. These have mainly been studied in other species, and in vitro (Sengel 1976^[8]; Woodley et al 1987), but it is likely that the general principles also apply in human development.

In the early stages of development the ectodermal/mesenchymal interactions contribute to the structuring of limb or facial primordia, e.g. the ectoderm promotes a chondrocyte free zone beneath it preventing chondrogenesis within the dermis and myogenic zones. Later, the dermis controls transformation of the ectoderm into epidermis, and regulates its basal–apical polarization, differentiation, and stratification, by maintaining controlled proliferation of the basal layer cells. The epidermis, in turn, induces the dermis to start morphogenesis. Complicated

interactions are involved in the morphogenesis of the epidermal appendages, e.g. hairs, scales, feathers, as revealed by intra-class and inter-class dermal–epidermal recombinations. These have shown that the presence or absence of appendages is due to a regional property of the underlying dermis, which also determines their type, distribution and pattern. The epidermis determines the class-specific morphology of appendages, their cephalocaudal polarization, and the species-specific amino acid composition of keratins. For example in the chick, when mesenchyme from the thigh is inserted beneath ectoderm that covers the proximal portion of an embryonic wing, the wing ectoderm forms leg feathers. In fact combination of mouse mesenchyme (which would normally cause the overlying ectoderm to form hair) with chick corneal epithelium (which would normally become curved and transparent) results in the first stages of feather formation. The ectoderm constructs the typical appendage of avian skin being unable to 'interpret' the mouse mesenchyme instructions to form the mammalian appendages—hair (Wessells 1977^[1]). Many 'informative' and 'permissive' messages and signals between epidermal cells and dermal cells and matrix are involved in these overall interactions. Matrix macromolecules including some of those of the basement membrane zone mentioned above, i.e. fibronectin, integrins, cell adhesion molecules (cadherins), and soluble factors such as nerve growth factor, epidermal growth factor, retinoids and cyclic nucleotides have been suggested as mediators. There is evidence that calcium is involved as signal or messenger for some of the cell–substrate and cell–cell adhesive interactions involved (Fairley 1991^[2]). Similar interactions are also involved in wound healing and remodelling (see pp. 412^[3], 416^[4]).

Cardiovascular System

Endothelial development is morphologically first evident in the *extraembryonic tissues*. Here angioblastic tissue differentiates from extraembryonic mesenchyme in three regions:

- in the splanchnopleure of the yolk sac
- in the body stalk (containing the allantois)
- in the somatopleure of the chorion (Hertig 1935^[5]; Bloom & Bartelmez 1940^[6]).

It is suggested that the earliest endothelial cells differentiate from mesenchyme derived from the *parietal hypoblast* (Enders & King 1988^[7]; see p. 140^[8]). In the yolk sac and base of the body stalk, small, more or less spherical groups of cells are found early in the third week, termed *blood islands* (3.158^[9]). Stages of transformation of islands into blood-containing *vessels* are controversial in detail, but it is widely believed that peripheral cells of the islands flatten as the vascular endothelium, while the central cells transform into primitive red blood corpuscles (3.158B^[10]). Later these small blood-containing spaces merge forming a continuous network of fine vessels. In the chorionic end of the body stalk and extraembryonic mesoblast lining the chorion typical blood islands are not found, but some mesenchymal cells give rise to solid strands of *angioblasts*. Each strand contains two or three cells with rod-shaped nuclei arranged in a single row, which soon develops a space occupied by one or more nucleated haemoglobin-containing cells. These spaces coalesce to form blood vessels which are lined by endothelial derivatives of the mesenchyme; the precise source of their contained blood cells is

uncertain. The earliest vessels, therefore, are formed at several separate centres; from the walls of these vessels buds grow out and become canalized, and thus converted into new vessels which join with those of neighbouring areas to form a close meshwork.

Intraembryonic Blood Vessels

These are first seen at the endoderm : mesenchyme interface within the lateral splanchnic mesenchyme at the caudolateral margins of the cranial intestinal portal. The origin of the intraembryonic angioblastic cells is not known, they may derive from migrating extraembryonic angioblastic cells, or a population of cells with angiogenic potential may be a product of one, or more, of the different types of intraembryonic mesenchyme. Chimeric experimentation has so far shown that all mesenchymal tissues, apart from notochord and prochordal plate, contain endogenous angioblastic cells, whereas no ectodermal tissues, i.e. neuroepithelium and neural crest mesenchyme, contain angiogenic cells (Noden 1991; see p. 156). Embryonic angioblasts are highly invasive, moving in every direction throughout embryonic mesenchymal tissue.

Prior to the establishment of the circulation, endothelial vessels are formed in two ways:

- by *vasculogenesis* (also termed *angioblastic vasculogenesis*), where new vessels develop in situ, e.g. endothelial heart tubes, dorsal aortae, umbilical and early vitelline vessels
- by *angiogenesis* (also termed *angiotrophic vasculogenesis*), where vessels develop by sprouting and branching from the endothelium of pre-existing vessels, e.g. as seen in most other vessel production.

Vasculogenesis has been subdivided into two successive phases (Poole & Coffin 1991); in type I, angioblasts arise in situ, as in the dorsal aorta; in type II, angioblasts migrate to the site of vessel development, as in the endocardium and postcardinal veins; angiogenesis continues from both these origins. (Early vascular patterns are described on pp. 222 and 230 et seq: see also 3.180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 3.179A,B.) Once initiated, concurrent with the onset of somite formation, the development of blood vessels progresses at a phenomenal rate. During this time the direction of blood flow through a vessel may reverse; thus it cannot be designated artery or vein until the other tunics (media and externa) have started to develop. In the head and neck, 'cardiac neural crest' cells, i.e. crest cells originating between the midotic placode and the caudal limit of somite three, contribute to the supporting layers of the developing endothelium, particularly the tunica media. In the trunk, local mesenchyme (probably splanchnopleuric) serves that function. The subsequent development of the blood corpuscles is described on page 1407.

Development of the Heart

Early Cardiac Development


In amniotes the heart is the earliest major organ to function. For obvious nutritive reasons it must not only accommodate a stream of blood but also begin to propel it. These early **functional** demands on the heart represent an important factor in the dynamics of its development. The early appearance of cardiac activity in the tubular hearts of chick and rat embryos was noted many years ago (e.g. Sabin 1920^[4] et seq; Goss 1942^[5]). First manifested by arrhythmic and sporadic ventricular contractions, these are rapidly superseded by regular peristaltic activity propagated unidirectionally along the cardiac tube. In the account that follows, attention is necessarily concentrated upon the complex changes which transform a tube into a chambered septate human heart. It is also necessary to keep in mind that the early heart is only a few hundred micrometres (μm) in size and that at every stage the heart must be an effective circulatory pump.

The heart is formed from at least three sources:


- *angioblastic mesenchyme* lateral to the cranial intestinal portal
- *midline splanchnopleuric coelomic epithelium*; this is ventral to the foregut endoderm after the head-fold stage
- *neural crest cells* derived from the region between the otic vesicle and the caudal limit of somite three.



These sources will produce respectively:

- the endocardium and cardiac mesenchymal cells which produce the valvular tissue of the heart
- the myocardium, including the conducting tissue of the heart, and the specific matrix proteins associated with the developing heart, i.e. the cardiac jelly
- the aorticopulmonary septum and the media of the great vessels, and, possibly contributes to the conducting tissue of the heart.

Primitive cardiac myocytes can first be seen in the unfolded embryo, during the pre- and early somite period, as simple, cuboidal epithelial cells of the splanchnopleuric coelomic epithelium superjacent to the endoderm. Subsequently, elongated and flattened angioblastic mesenchymal cells differentiate from mesenchyme between the myocardial cells and the underlying endoderm. These groups of angioblastic cells are amongst the earliest intraembryonic vascular precursors to appear. They arise as single cells at the ventrolateral edges of the cranial intestinal portal and subsequently aggregate to form an epithelium, the *endocardium*, enclosing small cavities. The endocardial lined spaces coalesce in the vicinity of the developing foregut to establish bilateral, hollow tubular structures (**3.159A,B** ). By stage 9, when the embryo has 3–4 somites, the head fold is apparent and the cardiac region of the embryo is undergoing reversal. The splanchnopleuric coelomic epithelium is now ventral to the foregut with the forming endocardial



tubes dorsal.

It is worth noting here that the epicardium, as seen in the adult heart, is not present at this stage. Cardiac myocytes differentiate from the splanchnopleuric layer of coelomic epithelium which passes from right to left (in the folded embryo), ventral to and in contact with the primitive foregut; this layer is continuous on each side with the splanchnic walls of the pericardio-peritoneal canals. The somatopleuric coelomic epithelium at this point gives rise to the *parietal pericardium*. There is no visceral pericardial layer (unlike, e.g. the formation of visceral peritoneum; see p. 174 ). The *epicardium* is sometimes included in descriptions of the myocardium as epimyocardium; however, the epicardial layer proper develops later from septum transversum mesenchyme cells which spread over the myocardial tube.

The bilateral endocardial tubes become connected, merging to form one endocardial tube almost completely surrounded by putative myocardial cells; these, unlike the endocardium, form a continuous epithelium across the midline from the outset (**3.1C,D** ). The endocardium is suspended by a primitive *dorsal mesocardium*. Using a monoclonal antibody specific for endothelial cell precursors (QH1), reactive cells have been demonstrated close to the basal surface of the endoderm just ventral to the foregut. It is suggested that this area may stabilize developing endothelium and promote fusion of the bilateral endothelial tubes. This portion of the endoderm remains in contact with the endocardium for some time via the dorsal mesocardium (Bolender & Markwald 1991 .

The two endocardial tubes fuse across the midline progressively commencing at the arterial end (outflow tract) and extending to the venous end where the two putative atria are, initially, widely separated from each other. The single heart tube so formed is divided into, caudorostrally, the prospective left ventricle, prospective right ventricle and the outflow tract (see below). By stage 10 (22 days), the heart is thus composed of *inner* and *outer epithelial tubes*, the endocardium and myocardium respectively. These tubes become separated widely by a basal extracellular matrix secreted by the myocardial cells.

Extracellular Matrix of the Heart

The extracellular matrix of the heart, historically termed *cardiac jelly*, promotes occlusion of the tubular lumen during contraction, thus providing mechanical assistance for the generation of a blood flow; it also acts as a site for the deposition of inductive factors from the myocardial cells which may modify the differentiation of specific endocardial cells. It has been termed a gelatinoreticulum, a *myoepicardial reticulum* (**3.159C,D** ) and more recently the *myocardial basement membrane* (Bolender & Markwald 1991 ); it is composed of, inter alia, hyaluronic acid, hyaluronidase and fibronectin (see below). Endothelia generally show great diversity; subtle differences in morphology such as the presence or absence of fenestrations, or the extent of tight junctions, support the concept of *regional specificity of endothelia*. Within the heart the endocardium exhibits regional diversity with respect to its development potential. Inductive signals originating from the myocardial cells cause a *subset* of endocardial cells lining the atrioventricular canal and the proximal outflow tract to *transform into mesenchyme* (*cardiac*

mesenchyme), while the endocardial cells in other regions of the heart, for example in the ventricle, remain epithelial (Bolender & Markwald 1991^[4]). When activated by myocardial inductive factors the endocardial cells lose their cell–cell associations, showing decreased expression of N-CAM (a cell adhesion molecule, see p. 111^[4]) and increased expression of substrate adhesion molecules such as chondroitin sulfate and fibronectin, they undergo cytoskeletal rearrangements necessary for migration, and they express type I procollagen. Specific myocardial inductive factors have not yet been identified; proteins known to be present in the matrix include hyaluronic acid, hyaluronidase, fibronectin and putative cardiac adherons. The latter when fractionated and reapplied to an endothelial monolayer results in decreased expression of N-CAM, an event occurring prior to endocardial transformation to mesenchyme in situ.

The transformation of endocardium to mesenchyme may, perhaps, be the only example of a mesenchymal population derived from an endothelial lineage (Markwald et al 1990^[4]); the cells uniquely retain expression of the endothelial marker QH1. It is believed that the transformation is triggered by an intrinsic clock as a similar change occurs in vitro when atrioventricular endocardium is cultured with myocardium.

Formation of cardiac mesenchyme cells at the atrioventricular canal and the proximal outflow tract is followed by their migration into the myocardial basement membrane. Accumulation of mesenchyme and matrix in these regions produces protrusions, the *subendocardial cushions*, which bulge into the primary heart tube and support the valve function of the atrioventricular canal and outflow tract. The eventual fusion of opposing cushion tissue across the lumen of the atrioventricular canal forms a wedge of mesenchyme that serves to guide the union of the internal muscular septa. Interestingly the position of the subendocardial cushions corresponds to the future positions of the fibrous skeleton of the heart and the valves (3.160^[4]).

Simple Heart Tube

The simple heart tube elongates and develops an asymmetric twist. Aided by the position of the subendocardial cushions and the ventral fold of the heart a succession of cavities, joined by more constricted regions, begin to define the sinus venosus, atrium, ventricle and bulbus cordis (3.161^[4], 162^[4], 163^[4], 164^[4]). Initially somewhat cylindrical, the cavities rapidly become more spherical (within 24 hours in the mouse). Coincidentally, cells of the myocardial mantle invade the subendocardial reticulum and form a complex network of intercommunicating *trabeculae*, external to but ultimately indenting and becoming clothed by endocardium. In many vertebrate groups the myocardium remains predominantly trabecular, but in birds and mammals compact layers of cardiac muscle develop external to the trabeculae. The latter also persist, however, but constitute a lesser volume of the propulsive tissue (see below).

These early events in cardiac development provide an approximate parallel between phylogeny and ontogeny. The evolution from a 'trabecular' heart to an organ with rounder chambers and a largely compacted myocardium is likely to be an expression of increased efficiency. An attempt to demonstrate this by mathematical analysis (Chalice & Viragh 1973^[4] et seq) provides some

corroboration and also an explanation for the persistence of the internal trabeculation in the mammalian heart.

For discussions and bibliographies concerning experimental studies and the importance of haemodynamic influences on regional cardiac morphogenesis, consult Stalsberg and De Haan (1968^[1]), Bellairs (1971^[2]), Balinsky (1981^[3]), Orts-Llorca et al (1982^[4]), Bockman & Kirby (1990^[5]), Feinberg et al (1991^[6]).



General Cardiac Development


The *dorsal aortae* arise in situ as paired endothelial vessels. They extend caudally into the body stalk, establishing continuity with the umbilical arteries, which precede them in time of appearance. At their cranial ends the dorsal aortae curve ventrally round the sides of the foregut to reach the pericardium and become continuous with the cranial end of the endothelial heart tube, thus forming the first pair of aortic arches (3.161^[7]). In all vertebrates in which the heart and aortae are laid down **before** the formation of the head fold, the arteries communicate with the *caudal* end of the heart. When the head fold forms, as noted, the ends of the heart are **reversed** and the cranial ends of the dorsal aortae are curved forwards round the sides of the foregut as the first aortic arches.


A transverse groove appears on the surface of the heart tube about its middle, the junction of the *bulbus cordis* with the *ventricle*. The bulbus is cranial to the groove and continues as the first pair of aortic arches. The ventricle shows a second groove at its caudal end where it opens into a *common atrium*, which, initially, is embedded in the floor of the pericardium (the future *septum transversum*) and the chamber is disposed transversely. On each side the common atrium is joined caudally by a short venous trunk, formed by the union of the corresponding umbilical vein with veins issuing from the *vitelline* (yolk sac) *plexus*. These trunks represent the right and left *horns* of the *sinus venosus* (*sinual horns*) so that the common atrium may justifiably be termed a *common sinuatrial* (or *sinoatrial*) *chamber*. The umbilical and vitelline radicles of each sinual horn are soon joined laterally by a *common cardinal vein* (each the confluence of a *precardinal* and *postcardinal vein*; see p. 321^[8]).

At this point it should be noted that the regions of the early heart tube have over the years received many, often confusing names. Keith (1924^[9]) described, from caudorostrally, atrial, ventricular, bulbar and truncal components of the straight heart tube; Streeter (1942^[10]), described atrial, ventricular and bulbar portions only, but subdivided the bulbus into right ventricle, conus cordis and truncus arteriosus; Anderson et al (1978^[11]) described the ventriculobulbar portion as possessing two segments only, the primitive ventricle and the bulbus cordis, but they divided the ventricle into three parts, the inlet part—related to the atrioventricular valve, the trabecular portion, and the outlet part—which supports an arterial valve; Teal et al (1986^[12]) have recommended that the terms bulbus, conus and truncus be avoided completely, referring to the region between the trabeculated part of the right ventricle and the aortic sac as the outflow tract. Unfortunately the literature continues to retain all manifestations of the terminology including

reference to the bulbus cordis/outflow tract as the conotruncus.

Early in the fourth week the heart tube undergoes a striking change. Hitherto the parietal pericardium has increased in length proportionately with the heart, but now the heart tube grows more rapidly and the bulboventricular tube bulges ventrally and caudally, forming a U-shaped loop; the bulbus cordis forming the right limb and the ventricle the left. The loop is conspicuous throughout the fourth and fifth weeks, and seen as a deep *bulboventricular sulcus* externally (3.161 ) and a corresponding *bulboventricular ridge* projects internally. Other factors often considered operative in determining the disposition of the loop are modifications in the tubular heart flow patterns, and coordinated ciliary action of the coelomic epithelium (Afzelius 1979 




Dorsolateral recesses of the splanchnopleuric pericardial layer adjacent to the myocardium deepen and approach one another (3.159D 


At stage 10 (3.179A 



Towards the end of the fourth week the connection between the bulbus cordis and the first pair of aortic arches lengthens to form the *truncus arteriosus*, and the cranial end of this vessel becomes connected to the dorsal aortae by a further five pairs of aortic arches. By this time the *venous drainage* of the body wall and neural tube has been established. On each side a *precardinal vein*, from the cranial end of the embryo, unites with a *postcardinal vein* from the

caudal region to form the *common cardinal vein (duct of Cuvier)*; the latter vessel opens close to the umbilical and vitelline veins into the dorsocaudal part of the common sinuatrial chamber (the three vessels on each side thus forming the right or left *sinual horn*).






As the chorionic circulation already exists the embryo can now exchange materials with the maternal blood in the intervillous space. This is not effected suddenly; initially the blood volume and its cellular content in the heart and vessels of the embryo is insufficient to enable it to take full advantage of this new source of nourishment, and until this is rectified, the embryo continues to draw upon the coelomic fluid.



The separation of a definitive *sinus venosus* from the *common atrium* completes the definition of the primitive chambers of the heart. A crescentic groove appears on the left wall of the sinuatrial chamber and rapidly deepens to the right. Hence the left horn of the sinus venosus loses its connection with the left part of the atrium and becomes linked to the right sinual horn by separation of the caudal part of the sinuatrial chamber; the latter now constitutes the *body of the sinus venosus*. At the same time the right sinual horn becomes more clearly demarcated from the right part of the atrium by a shallow groove, and its wide connection with the atrium (3.163 ) becomes relatively smaller (Foxon 1955 ). The right and left parts of the atrium grow cranially to occupy the dorsal part of the pericardial cavity, and later they bulge forwards, embracing the sides of the bulbus cordis (3.165 


The embryo has now reached a length of nearly 4 mm (3.179B ). It possesses 28 somites and has almost completed the fourth week of development. From this stage onwards it is more convenient to deal with the individual chambers with only occasional reference to the development of the heart as a whole.


It must be noted that the above account of early cardiogenesis, though widely subscribed to, is not without its critics. In considering the many factors governing cardiac development—phylogenetic, ontogenetic, and physiological—the last is usually underestimated and the first is perhaps stated too dogmatically (Foxon 1955 ). Ontogenetic mechanisms must conform to the early demand for a functioning heart, and cardiogenesis is not necessarily a mere repetition of phylogenetic steps, which are themselves uncertain, however plausible they may seem (De Vries & Saunders 1962 

Sinus Venosus

The right sinual horn increases rapidly in size at the expense of the left, due to the changes already outlined and to those occurring in the originally symmetric arrangement of the umbilical and vitelline veins by the development of the liver (3.186 , 187 , 188 , p. 322 ). As a result the vitello-umbilical blood flow enters the right horn through a wide but short vessel, the *common hepatic vein*, which becomes the cranial end of the inferior vena cava. In addition, the right horn receives the right common cardinal vein (from the body wall of the right side) and the body of the sinus, which conveys the blood from the left horn and left common cardinal vein. Later, when *transverse connections* are established between the cardinal veins (3.189 , 190

, the blood from the body wall of the left side reaches the heart via the veins of the *right* side. The left common cardinal vein then becomes much reduced in size and forms the oblique vein of the left atrium and the fold of the left caval vein, while the left horn and the body of the sinus venosus persist as the *coronary sinus* (3.189B )

The right sinuatrial horn opens into the right atrium through its dorsal and caudal walls. The orifice, elongated and often slit-like, is guarded by two muscular folds, the *right* and *left sinuatrial (venous) valves (valvules)* (3.165 )


These two valves meet cranially and become continuous with a fold which projects into the atrium from its roof, the *septum spurium*. Caudally the valves meet and fuse with the dorsal endocardial cushion of the atrial canal. The cranial part of the right sinuatrial valve loses its fold-like form, but its position is indicated in the adult heart by the crista terminalis of the right atrium; its caudal part forms the valve of the coronary sinus and most of the valve of the inferior vena cava. The medial (or left) end of the valve of the inferior vena cava is formed by a small fold continuous with the dorsal wall of the sinus venosus, the *sinus septum*. The latter intervenes between the orifice of the common hepatic vein and the opening of the body of the sinus. (In the mature heart see the *tendon of Tondaro* and *triangle of Koch*, p. 1477 )





The left venous valve blends with the right side of the atrial septum and usually no trace of it can be seen in the adult heart.





As the sinuatrial valves undergo these changes the right sinuatrial horn becomes incorporated in the right atrium and expands to form its smooth dorsal wall, medial to the crista terminalis. This part of the adult atrium is termed the *sinus venarum*, the receiving chamber of the large venous orifices. The right half of the primitive atrium forms the internally ridged, more muscular, wall anterior to the crista terminalis and the right auricular appendage.

Right and Left Atria

As stated, the common atrium is derived from the cranial part of the sinuatrial chamber. It receives the opening of the sinus venosus dorsocaudally and to the right of the median plane, while it communicates ventrally with the ventricle through the atrioventricular canal, which has resumed its median position by the middle of the fifth week, thus permitting both right and left parts of the atrium to communicate with the common ventricular cavity. Dorsal and ventral swellings appear in the walls of the atrioventricular canal between the endothelial tube and the myoepicardial mantle. These, the *atrioventricular endocardial cushions*, consist of a core of myocardial basement membrane matrix and mesenchymal cells derived from the endocardium. They encroach on the canal and eventually fuse, leaving a relatively small orifice on each side. The fused tissue constitutes the *septum intermedium* (of His), which separates the two small right and left atrioventricular orifices and canals.



Internal separation into right and left atria is mainly effected by sequential growth of two septa (but with additional, less prominent structures). First the *septum primum* grows from the dorsocranial atrial wall as a crescentic fold (3.166A ) , separated from the left sinuatrial valve

by the *interseptovalvular space*. The ventral horn of the crescent reaches the ventral atrioventricular cushion, the dorsal horn the dorsal cushion. Ventral and dorsal refer to the positions of the cushions after the atrium repositions to lie dorsal to the bulbus cordis. Strictly the cushions are ventrocranial and dorsocaudal in position, but ventral and dorsal are in general use and will be retained here. Ventral and caudal to the advancing edge of the septum the two atria communicate through the *foramen primum* (3.166A ). Free passage of blood from right to left atrium is essential throughout fetal life, as oxygenated blood from the placenta reaches the heart via the inferior vena cava (pp. 1500 , 1503 ); therefore, as the foramen primum diminishes, the septum primum breaks down dorsally and a new right–left shunt, through the *foramen secundum*, is formed before the end of the fifth week. The foramen primum is finally occluded by fusion of the edge of the septum primum with the fused atrioventricular cushions, in the median plane. The foramen secundum enlarges, allowing sufficient free passage of blood from right to left atrium (3.166 ) , and it persists throughout intrauterine life as *interatrial septal complex* (see below). At first the foramen secundum is sited craniodorsally in the septum primum but it becomes modified until it is cranioventral.




Towards the end of the second month the muscular wall of the atrium becomes invaginated as another crescentic septum on the right side of the septum primum (3.166B,C ). This, the *septum secundum*, involves more than the whole width of the interseptovalvular space; thus the dorsal attachments of the septum primum and the left sinuatrial valve are carried into the interior of the atrium on its left and right surfaces respectively. The superior (ventrocranial) and inferior (dorsocaudal) horns of the septum secundum at first grow ventrally; the superior horn grows much more rapidly and fuses first with the septum intermedium; it is then continuous with the *sinus septum* (see above). Thus the free edge of the septum secundum (*crista dividens*) is at first directed caudoventrally and later caudally alone; it overlaps the foramen secundum (3.166C,D ); thus the septum primum acts as a flap valve. Since the blood pressure is greater in the right atrium than in the left, the blood flows from right to left, but not conversely. The right–left flow occurs through the 'true', but somewhat misnamed *foramen ovale*, proceeding from the right atrium under the crescentic free border of the septum secundum, thence through the oblique cleft between the (parted) secondary and primary septal surfaces, to finally enter the left atrium through the foramen secundum. After birth the intra-atrial pressures are equalized and the free edge of the septum primum is therefore kept in contact with the left side of the septum secundum and fusion occurs. Not infrequently the fusion is incomplete, but the remaining cleft is usually small, valvular and has no functional significance. The initially free, crescentic margin of the septum secundum forms, after fusion, the *limbus fossae ovalis* and the septum primum the floor of the *fossa ovalis* of the adult heart. An alternative derivation of the septum secundum from a ridge developing to the right of the line of fusion between the confluent endocardial cushions and the septum primum has been advanced (Odgers 1934 ). The dorsal horn of the septum secundum is said to incorporate tissue derived from part of the left sinuatrial valve; its contribution, however, remains uncertain; sometimes, small vestigial remnants persist to maturity. Another view embodies the above suggestion, but regards the valve as of minor importance in this connection, describing, however, yet another ridge—the *septum accessorium*—contributing to the lower part of the dorsal border of the limbus (Christie 1963 ).

Early in the development of the septum primum a single, common *pulmonary vein*, suggested to

develop from angiogenic cells positioned in the early dorsal mesocardium but in continuity with the endoderm, opens into the caudodorsal wall of the left atrium close to the septum. It is the union of a right and a left pulmonary vein, each formed by two small veins issuing in turn from each developing lung bud. Subsequently the common trunk and the two veins forming it expand and are incorporated in the left atrium to make up the greater part of its cavity. This expansion usually continues as far as the orifices of the four veins, which thus open separately into the left atrium; variations, however, are quite common. The left half of the primitive atrium is progressively restricted to the mature auricular appendage.


During the second month the two atria bulge ventrally one on each side of the bulbus cordis, which lies in a groove on their ventral surface (3.162 , 165 ). These projecting parts of the atria form the auricular appendages of the adult heart.




Ventricles, Bulbus Cordis and Truncus Arteriosus

The process of separation of the ventricles is intimately related to that of the aortic and pulmonary orifices at the distal end of the bulbus (3.167 , 168 , 169 ) and also to the division of the truncus arteriosus into pulmonary and aortic channels. Their interdependence is such that the history of the truncus arteriosus is dealt with here, although strictly it takes no part in the formation of the heart itself. Bulbotruncal separation is conveniently considered before final interventricular septation and valve modelling. As intimated these complex events are the result of mutual interaction between factors controlling pattern formation, differential growth and the continuously changing blood-flow paths, volumes and pressures. This interplay moulds the grooves, ridges, outpouchings, valve complexes and varying myocardial thickness and architecture. Although described sequentially, many of these events occur simultaneously.

Blood enters the bulboventricular cavity through the right and left atrioventricular canals (ventricular *inflow tracts*) and is ejected through the proximal and distal bulbus (*outflow tracts*). Blood flow from the future left ventricle passes obliquely to the dorsal part of the bulbus, whereas right ventricular blood has a reverse inclination to the former and is expelled through the ventral part of the bulbus. These inclinations impose a mutually spiral flow on the two streams as they traverse the truncus.

Four endocardial cushions—ventral, dorsal, right and left—form in the distal part of the bulbus and the right and left cushions fuse to constitute a *distal bulbar septum*. This separates a ventral, *pulmonary orifice* from a dorsal, *aortic orifice*, and later the cushions divide and become modified to form the *semilunar valves* (see below).




The separation of the pulmonary trunk from the aorta is a more complicated process. Two ridge-like thickenings project into the interior of the truncus arteriosus between the entwined spiralized streams of blood. Proximally, the ridges project from the **lateral** walls of the vessel but, progressing distally, the right ridge passes obliquely on to the **ventral** and then the **left** wall, while the left ridge extends on to the **dorsal** wall and then the **right** wall (3.168 ). The ridges are therefore spiral and their fusion forms the *spiral aorticopulmonary septum*. Proximally this






meets and fuses with the distal bulbar septum, and in accord with its spiral form the pulmonary trunk, which lies ventral to the aorta at its orifice, curves round to its left side as it ascends and finally lies dorsal to it (3.168 ). Distally the aorticopulmonary septum meets the dorsal wall of the aortic sac (see p. 312 ) cranial to the point where it is joined by the sixth pair of aortic arches, and thus the latter become branches of the pulmonary trunk while the remaining arches retain communication with the aorta (3.168 .

The separation of the two ventricles from each other leaves the right ventricle in communication with the right atrium (inflow tract) and the pulmonary artery (outflow tract), and the left ventricle in communication with the left atrium (inflow tract) and the aorta (outflow tract). It involves a series of complex changes in which three distinct factors contribute to the formation of the *adult ventricular septum*:

- the *fetal ventricular septum*
- the *proximal bulbar septum*
- the *atrioventricular endocardial cushions*.




Fetal Ventricular Septum

During the fifth week the right and left definitive ventricles appear as slight projections on the external surface of the primitive common ventricle. It is uncertain whether the right definitive ventricle is solely a derivative of the common ventricle, or of the caudal end of the primitive bulbus, or of both. In either event, the appearance of a caudal crescentic ridge in the inside of the heart indicates the separation between the two ventricles and, as the heart enlarges, this ridge deepens to form the early ventricular septum. The dorsal and ventral horns of the septum grow along the ventricular walls to meet and fuse with the corresponding endocardial cushions of the atrioventricular canal near their **right** extremities (3.167 ). The septum has a free sickle-shaped margin which, with the endocardial cushions, bounds a circular *interventricular foramen* (3.168 ) (sometimes delineated temporally as the *interventricular foramen primum*; 3.170 .




At first the bulboventricular junction is marked by a distinct notch on the outside of the heart (3.161 ) and inside is a corresponding *bulboventricular ridge*. The latter is between the atrioventricular orifice and the caudal part of the bulb (3.167 ) and its absorption is essential for the development of a four-chambered heart. Partly by absorption of the bulboventricular ridge and partly growth of the atrioventricular region, the **right** extremity of the atrioventricular canal comes to lie **caudal** to the orifice of the bulb (3.168 ). This alteration in relative positions of the structures concerned occurs while the ventricular septum is forming, paving the way for completion of ventricular partition (Wenink 1971 , 1976 .

Proximal Bulbar Septum

The proximal bulbar septum separates the bulbus cordis into pulmonary and aortic channels

(*ventricular outflow tracts*), and is formed by the *right* and *left bulbar ridges*, which are in continuity with the corresponding distal bulbar endocardial cushions (which form the *distal bulbar septum*—see above). The ridges appear broad, shallow and less defined at their proximal and distal ends, but are somewhat taller and better defined in their midportion (3.170 ). The right bulbar ridge grows across the dorsal wall of the bulb and right extremity of the fused atrioventricular endocardial cushions to reach the dorsal horn of the free, crescentic edge of the ventricular septum and obliterates the ventral or cranial part of the right atrioventricular orifice (3.168 ). The left bulbar ridge crosses the ventral wall of the bulb to reach the ventral or cranial horn of the ventricular septum. The bulbar ridges fuse thus separating the conus arteriosus of the right ventricle from the aortic vestibule; however, the caudal edge of the bulbar septum is still separated from the free crescentic edge of the ventricular septum by a diminishing interventricular channel (this has been termed the interventricular foramen secundum). The latter is closed by growth of tissue from the right extremity of the fused atrioventricular cushions (Odgers 1938 ) and this fuses, on its one aspect with the caudal border of the proximal bulbar septum and on its other with the margin of the ventricular septum. (A transitory interventricular foramen tertium has been described; it can be seen towards the end of the sixth week of gestation as an orifice 80 µm in its largest diameter. A dimple less than 40 µm in diameter is left for a brief period of time at the site of closure on the endocardial surface of the left ventricle.) The dorsal part of the bulb largely becomes absorbed, but its position is indicated by the dorsal wall of the aortic vestibule, which, however, is mainly formed by tissue extensions from the fused atrioventricular endocardial cushions.

Atrioventricular Endocardial Cushions

At their time of fusion the atrioventricular endocardial cushions are large relative to the size of the atrioventricular orifices. The atrial septal complex meets the approximate centre of the atrial surface of the cushions; the ventricular septum, however, meets them near their right margins. Thus a part of the fused cushions intervenes between the *right atrium* and the *left ventricle*, and it is this which forms the right wall of the aortic vestibule (*atrioventricular septum*, see 9.34 ). The *membranous* part of the *interventricular septum*, continuous dorsally with the membranous *atrioventricular septum* in the completed heart (3.171 ), is also formed by proliferation of cushion tissue from the right extremity of the fused atrioventricular cushions. The persistence of an interventricular communication may follow anomalous development in this region. It may be noted that it is the craniodorsal part of the bulbar orifice, which lies above the ventricular septum, and normally becomes incorporated in the aortic vestibule through which the left ventricle discharges into the truncal aortic channel. In some cases of persistent interventricular foramen the aortic orifice is described as 'overriding' the free upper border of the muscular interventricular septum (p. 1503 .

Development of the Fibrous Skeleton of the Heart


The *valve complexes of the heart*, four in number, arise in two main cardiac zones:

- the aortic and pulmonary valves at the distal bulbotruncal junction
- the mitral and tricuspid complexes extending from their inception between the



atrioventricular junctions and loci on the interior of the ventricular walls.


Each commences as an internal endocardial projection of varying form enclosing cardiac mesenchyme (myocardial basement membrane matrix and mesenchymal cells). In some regions the mesenchymatous cells proliferate, transform into fibroblasts and produce a geometrically organized collagenous framework that varies with site and functional demands. Elsewhere the core is invaded by differentiating cardiac myoblasts.

Atrioventricular Valves


These develop as shelf-like projections from the margins of the atrioventricular orifices, directed as almost complete conical sheets towards the ventricles, their advancing edges continuing, initially as trabecular ridges, deep into the ventricular cavity. With continued differential growth and excavation on their ventricular aspects, each sheet develops two (mitral) and three (tricuspid) marginal indentations, defining the principal *valve leaflets*, minor marginal indentations (clefts) subdividing some leaflets into scallops. Each leaflet develops functionally significant regional variations in surface texture; its core condenses as a collagenous lamina fibrosa. The latter blends at its atrioventricular base with the inappropriately named fibro-areolar valve 'annulus'—each a part of the complex, functionally crucial, fibrous 'skeleton' of the heart. The anterior leaflet of the tricuspid valve and both the anterolateral and posteromedial leaflets of the mitral valve appear at about the time when fusion of the atrioventricular cushions and bulbar ridges takes place. Delamination of the septal leaflet of the tricuspid valve, however, occurs after closure of the interventricular foramen during the seventh to eighth week of gestation (3.172 ).

Embryonic Trabeculae


These start to emerge in the apical endocardial region of the primitive ventricles during stage 15 (32 days gestation). By stage 17 (42 days) well-developed embryonic trabeculae show a typical spatial orientation creating a number of ventricular sinuses and giving a sponge-like appearance to the internal relief of both ventricles (3.173 ). *Definitive trabeculae* are first observed about the 40th day of gestation, appearing initially in the walls of both ventricles at the level of the atrioventricular junction; they develop towards the apex of the heart (3.174 ). By 10 weeks gestation the trabeculae are fewer and confined to the apical region where they gradually disappear following a process of simplification, deletion and reabsorption. The remodelling process is accomplished without the intervention of macrophages or inflammatory cells in the immediate interstitium. Mesenchymal tissue surrounding the trabeculae passes between the margins of the valve leaflets, indentations, clefts and defined zones of the leaflet surface as the white, glistening, compacted, collagenous *chordae tendinae* and these converge towards the tip and sides of the single or grouped *papillary muscles* and blend with their connective tissue framework. The muscles are the ventricular ends of the original embryonic trabeculae and, whilst free throughout their length, their mural ends are confluent with mural ventricular musculature and receive a dense population of its nerves and specialized conducting tissues. (For details of the disposition, architecture and some functional implications of the cardiac valve complexes


and fibrous skeleton see pp. 1481-1489)


Aortic and Pulmonary Valves

These are formed from the four endocardial cushions which appear at the distal end of the bulbus cordis. The completion of the distal bulbar septum results in division of each lateral cushion into two; thus the number of thickenings is increased to six: three associated with the pulmonary orifice and three with the aortic. These are the rudiments of the aortic and pulmonary valves. Each cushion-derived intrusion grows and is excavated on its truncal aspect to form a semilunar valve cusp. Similar events affect the adjacent truncal or septal wall. Thus the pouches between the valves and the walls of the vessels gradually enlarge and form their related *sinuses*. The core of each cusp forms a collagenous lamina fibrosa, delicate and thin in each crescentic lunule, thick and compact in the central nodule, with marginal radiate and basal bands. The latter blend with the complex, scalloped, mural valve ring. Initially, one cusp of the pulmonary valve lies anteriorly and the other two posterolaterally, whereas one cusp of the aortic valve lies posteriorly and the other two anterolaterally. However, a rotation of the heart to the left before birth changes the orientation of the cusps of the pulmonary and aortic valves and this is reflected in the various schemes for the designation of these cusps in the mature heart (see pp. 1482-1488)



Development of Cardiac Muscle


Cardiac myocytes differentiate from the splanchnic coelomic cells of the pericardium. Myogenic activity begins at the beginning of stage 10, approximately 22 days gestation, when the embryo has about 4 somites. At this time the presumptive cardiac myocytes express myosin, actin, troponin and other contractile proteins. The cardiac myocytes do not fuse with their neighbours to form a syncytium as in skeletal muscle; rather they remain mononucleated, branched cells connected via intercellular junctions. For a detailed description of the development of cardiac muscle see page 770.

Concurrent with development of the contractile proteins of cardiac muscle, cardiac myocytes develop numerous *specific heart granules* which contain substances shown to induce natriuresis and diuresis, and a family of polypeptides generally known as *atrial natriuretic peptides*. Specific heart granules develop from the Golgi apparatus in both atria and ventricles during fetal life but become restricted to atrial muscle in the adult (Challice & Viragh 1973)

Atrial natriuretic peptide is measurable when the heart is recognizably four-chambered. Within the atria almost all cells are capable of its synthesis (Navaratnam et al 1989)

Development of the Conducting System of the Heart

The development of the conducting system has been difficult to elucidate due to the inability of conventional histological staining methods to identify and delineate conducting tissues from other cardiac components during development. However, the recent descriptions of the patterns of expression of a number of markers for the conducting system such as HNK-1 (Nakagawa et al 1993) (which is identical to Leu-7; Ikeda et al 1990)

, GIN2 (Wessels et al 1992)

neurofilament (Gorza & Vitadello 1989^[1]), connexin-43 (van Kempen et al 1991^[2]) and *Msx-2* (*Hox-8*) (Chan-Thomas et al 1993^[3]) have brought more clarity to this field.

In the formed heart two types of myocardium have been distinguished. First, the *working myocardium* of atria and ventricles which is specialized in contraction. Second, the *conducting system* which is specialized in the coordinated propagation of the impulse over the myocardium (see p. 1496^[4] for its precise anatomy in the adult). The conducting system comprises: the *sinuatrial node*, where the impulse is generated, the *atrioventricular node*, responsible for the delayed transmission of the impulse from the atria to the ventricles, and the *atrioventricular bundle* and *left and right bundle branches*, by which the impulse is rapidly spread over the ventricles. This differentiation in the nomenclature between contracting and conducting cardiac myocytes has focused attention on morphological evidence for different cell lineages, yet the embryonic heart has a measurable electrical activity, as seen by electrocardiographic measurements (ECG), with no distinct conducting system. (For recent reviews see Lamers et al 1991^[5]; Moorman & Lamers 1992^[6]).

Development of the Nodes

The early heart tube is not segmented and, although a polarity can be observed along its craniocaudal axis, it is in essence a homogeneous tissue. Pacemaker activity is seen in the inflow tract from the earliest time (van Mierop 1967^[7]). Initially there is a poorly coupled pattern of excitation and contraction but this rapidly becomes a rhythmic activation pattern (Kamino 1991^[8]). Impulses so generated are *slowly propagated* leading to a peristaltoid form of contraction that is characteristic of the tubular heart (Patten & Kramer 1933). Indeed the slow propagation of the impulse is the predominant functional feature which distinguishes the myocardium of the early heart tube from that of the more advanced stages, leading to its description as *primary myocardium* (Moorman & Lamers 1992^[9]). It is suggested that the primary myocardium will give rise to both working myocardium and the conducting system (Patten 1956^[10]; Moorman & Lamers 1992^[9]), although some support the view that these myogenic lineages are separate and arise from different cell lines. Neural crest has been suggested to give rise to the conducting system, in some part due to the expression of HNK-1 and neurofilament in the conducting tissue (Gorza et al 1988^[11]). However, sinus node activity can be demonstrated prior to crest cell arrival in the heart and the expression of such markers may reflect the ambiguous neural and myocardial properties of the conducting system.

As development proceeds, segments of *fast-conducting* atrial and ventricular working myocardium differentiate within the slowly conducting primary myocardium (Arguello et al 1986^[12]; de Jong et al 1992). The resulting heart consists of five segments (3.175^[13]) displaying alternately slow and fast conduction; this is also reflected in the alternating levels of the major cardiac gap-junctional protein connexin-43 in the consecutive segments (van Kempen et al 1991^[14]) and the appearance of an electrocardiographic output (van Mierop 1967^[7]). This architecture permits the pumping function of the embryonic heart in which no valves are present: the slowly conducting segments between the atrium and ventricle (atrioventricular canal), and ventricle and great arteries (outflow tract), contain the endocardial cushions which function as sphincteric valves. The segments persist until one-way valves have been sculpted from the endocardial

cushions. The primary myocardium of the outflow tract regresses along with the formation of the semilunar valves and has virtually disappeared around the twelfth week of human development. The primary myocardium of the atrioventricular canal will become incorporated into the atria upon the formation of the atrioventricular valves from the ventricular inlets and the annulus fibrosus between 6 and 12 weeks of development; some persists as the, still slowly conducting, atrioventricular node.

Rings of *cardiac specialized tissue*, precursor tissue of the conducting system in the formed heart, have been thought to be present in the embryonic heart at the sinuatrial, atrioventricular, bulboventricular (interventricular) and bulbotruncal junctions (Wenink 1976^[1]; Anderson et al 1974^[2]), as in the *four ring theory*'. However, it is crucial to realize that the primary myocardial, slowly conducting segments that remain after the formation of the atrial and ventricular segments should not be considered as newly formed rings. The presence of an *interventricular ring* has been immunohistochemically confirmed; it gives rise to the fast-conducting ventricular part of the conducting system encompassing the atrioventricular bundle and bundle branches (see below). Thus the conducting system of the formed heart encompasses two distinct functional components: the slowly conducting nodal component, consisting of persisting primary myocardium of the flanking segments, and the fast-conducting ventricular component, comprising the atrioventricular bundle and bundle branches.

The concept of cardiac specialized tissue may unintentionally suggest that it is a homogeneous tissue with a single function, being more specialized than other myocardium. However, it is contradictory to suggest that the nodal tissue which is reminiscent of primary myocardium is more specialized than the well-differentiated working myocardium.

Although since the discovery of the nodes attempts have been made to identify *internodal tracts* of specialized atrial cells (for review see Janse & Anderson 1974^[3]), as yet there is no convincing evidence to substantiate the presence of such tracts. Preferential conducting pathways in certain areas of the atrium can be accounted for by regional differences in the histological architecture and geometry of the atrial walls and septum (but see also page 1500^[4]).

Development of the Ventricular Conducting System

The development of the ventricular conducting system appears to have become essential, with the evolutionary emergence of two ventricles, to guarantee simultaneous contraction of both ventricles. Hence the development of the ventricular conducting system is obligatorily associated with ventricular septation. In the early human embryo (stage 14, 5 weeks) (Wessels et al 1992^[5]; Ikeda et al 1990^[6]) and chick embryo (Chan-Thomas et al 1993^[7]) a myocardial ring can be identified encircling the foramen between the presumptive left and right ventricles on top and astride the developing ventricular septum (3.176^[8]). At this stage of development the atria are connected to the left ventricle only. The interventricular ring is a ventricular structure which, in the inner curvature of the ring, is also part of the myocardium of the atrioventricular canal. Between stages 16 and 19, as a result of the rightward expansion of the atrioventricular canal during subsequent stages, the right atrium gains access to the right ventricle, while, as a result of

an apparent leftward expansion of the outflow tract, the left ventricle gains access to the subaortic outflow tract (3.177, 178). This entire process can be visualized by the expression of GIN2, one of the neuronal markers of cardiac myocytes. The atrioventricular bundle develops from the *dorsal portion of the interventricular ring* and is contiguous with the left and right bundle branches in the top of the ventricular septum. The anterior portion of this ring has been called the *septal branch*. This so-called 'third branch' of the atrioventricular bundle has been described as a 'dead-end tract' in some malformed hearts (Kurosawa & Becker 1985). It is not yet clear whether the GIN2-positive ring contributes to the formation of the atrioventricular node. As a consequence of the rightward expansion of the atrioventricular canal part of the GIN2-positive ring encircles the *right atrioventricular junction* and will end up in the lower rim of the atrium; this part of the ring is called the *right atrioventricular ring bundle* and has been demonstrated in fetal human hearts (Anderson & Taylor 1972; Anderson et al 1974). As a consequence of the apparent leftward expansion of the outflow tract, the GIN2-positive ring becomes positioned at the root and behind the subaortic outflow tract. This part is called the *retroaortic branch*. The septal branch, retroaortic branch and right atrioventricular ring bundle will disappear during normal development in mammals. 3.178 represents the entire system in the adult heart. As a rule basic processes are conserved in evolution. The presence of the entire system in adult chicken heart (Davies 1930) constitutes strong support for the unitary concept outlined.

The appreciation that the ventricular conducting system originates from a single interventricular ring provides a solid base to the understanding of the disposition of the conducting system in a number of congenital malformations. The concept accounts particularly well for the morphology and disposition of the atrioventricular node and bundle in hearts with straddling tricuspid valves, with double inlet left ventricles and with tricuspid atresia. The morphology of the latter heart defect is remarkably similar to the embryonic condition.

Fetal Heart Prior to Birth

The development of the chambers of the heart has now been traced to a stage at which the main features of the adult heart are established. It is to be noted that the pattern has developed in such a way as to provide for the sudden establishment of the pulmonary circulation at birth (Dawes 1961, 1969), although it is adapted to the persistence of the placental circulation for the remainder of fetal life. The presence of the ductus venosus (p. 324) ensures that a substantial proportion of umbilical oxygenated blood gains the right atrium with a limited loss of oxygen to the liver. However, some umbilical blood and portal venous blood enters the hepatic sinusoids through *venae advehentes*; their drainage through *venae revehentes* (eventually the grouped hepatic veins) returns the blood to the hepatic segment of the inferior vena cava, some admixture occurring here. It was claimed that only minor further admixture of relatively oxygenated and deoxygenated blood occurs in the right atrium (Barclay et al 1939) and that nearly all the oxygenated blood passes through the foramen ovale into the left atrium, so gaining the left ventricle, aorta and systemic circulation. However, there is evidence for the opposing view that there is considerable mixing of the superior and inferior vena caval streams in the right atrium (Born et al 1954; Lind & Wergelius 1954). The inferior vena caval blood is directed by its valve towards the cleft ('foramen ovale') in the atrial septal complex. It has been estimated that

about 75% passes through to the left atrium; the remainder mixes with the deoxygenated blood from the superior vena cava; separation occurs at the crista dividens of the septum secundum. Because the transition from a placental to a pulmonary circulation occurs suddenly at birth, the right ventricle and the pulmonary trunk of the fetus are relatively large, although only a small amount of blood passes through the lung. Most of the blood expelled by the right ventricle to the pulmonary trunk passes through the ductus arteriosus to the descending aorta and therefore is under higher pressure than blood in the aorta. Thus the muscular wall of the right ventricle is thicker than the wall of the left, a condition which persists throughout fetal life but is progressively reversed postnatally. The origin of the carotid and subclavian arteries from the aorta above the junction with the ductus arteriosus may be correlated with the relatively rapid growth of the brain, demanding a copious blood supply, with the more advanced development of the upper limbs, relative to the lower, at birth, and the overall cephalocaudal gradient in the growth of the trunk.

Embryonic Circulation

In early development the arteries of the embryo are disproportionately large and their walls consist of little more than a single layer of endothelium. The cardiac orifices are also relatively large and the force of the cardiac contraction is weak. As a result, despite the rapid rate of contraction, the circulation is sluggish, but this is compensated for because the tissues are able to draw nourishment, not only from the capillaries but also from the large arteries. As the heart muscle thickens, compacts and strengthens, the cardiac orifices become both relatively and absolutely reduced in size, the valves increase their efficiency and the large arteries acquire their muscular walls and they too undergo a relative reduction in size. From this time onwards the embryo is dependent for its nourishment on the expanding capillary beds and henceforth the larger arteries' function becomes restricted to controllable distribution channels to keep its tissues constantly and appropriately supplied.

It will be noted that the heart commences to beat early, prior to the development of the conducting system, and that a circulation is established before a competent valvular mechanism.

It has been stated previously (see above) that the heart must meet the functional demands of the embryo as well as follow its appropriate developmental pathways, such as the physiology of the cardiovascular system and its regulation during development which is of particular importance. It is beyond the scope of this book to address this issue in detail; however, the following points are relevant to an appreciation of cardiovascular development.

In the early embryo growth is exponential, with the embryo doubling its weight about every 4 hours. This geometric growth needs a concomitant growth and increase in efficiency of the cardiovascular system to supply nutrients and oxygen, and to remove metabolic waste products, especially in regions of active growth and proliferation.

Cardiac output increases in proportion with the weight of the embryo. Cardiac rate increases


with development; however, most of the increase in cardiac output results from a geometric increase in stroke volume. Noticeably, when dorsal aortic blood flow is matched to embryonic weight, blood flow remains constant over a more than 150-fold change in mass of the embryo. (For a discussion of early haemodynamics see Clarke 1991^[4].)

Further Development of the Blood Vessels

It is the case that the endodermal tissues, yolk sac (continuous with the splanchnopleure) and allantois, are primarily *vascular*, whereas the ectodermal tissues, chorion and amnion (somatopleure), are primarily *avascular*. The yolk sac and allantois secondarily vascularize the chorion in various ways, giving rise to the diverse varieties of placentae. Until recently it was not thought that any tissue interaction would be necessary for the process of intraembryonic vasculogenesis. However, evidence now points to a permissive interaction between *endodermal* epithelia and mesenchymes that are *angioblastic*, i.e. capable of differentiating into endothelia in situ, for the initial production of the early blood vessels. Such angioblastic competence has been demonstrated among the ventral mesenchymes (splanchnopleuric) with which the endoderm interacts. Dorsal mesenchymal populations (which include somites and somatopleuric mesenchyme) are more likely to be vascularized by budding from established endothelia (an angiotrophic mechanism; Sherer 1991^[4]). Once the mechanism of angiogenesis has been determined by the presence of endoderm or not, the timing of endothelial differentiation is controlled by the angiogenic cells. The ultimate pattern of vessels formed is controlled by the surrounding, non-angiogenic, mesenchyme (Noden 1991^[4]) and blood vessels become morphologically specific for the organ in which they develop; they also become immunologically specific, expressing organ-specific proteins.




During embryonic *vasculogenesis* (angioblastic vasculogenesis) and *angiogenesis* (angiotrophic vasculogenesis, see p. 299^[4]), changes occur in the vascular extracellular matrix. All blood vessels seem to be initially surrounded by a fibronectin-rich matrix which is later incorporated into the basal lamina along with inter alia laminin, a particularly early constituent (Risau 1991^[4]). Several layers of fibronectin-expressing cells can be seen around the larger vessels (e.g. dorsal aortae).


Major restructuring changes take place during the early development of the circulation. Anastomoses appear and disappear, capillaries fuse and give rise to arteries or veins, and the direction of blood flow may reverse several times. It is suggested that the *mechanical stresses* that endothelial cells have to withstand cause the development of the larger vessels (Risau 1991^[4]). The *tunica media* of the vessels appears after a stable vascular pattern has formed. Thus medial differentiation of the dorsal aorta starts in that part where major vessels are connected. Generally, the endothelium does not synthesize a basal lamina in those regions where remodelling is active and similarly the mesenchyme around such endothelium does not express α -actin, laminin, etc. Appearance of these molecules is indicative of cessation of branching and differentiation of the media. It is unknown how pericytes and smooth muscle cell differentiation is induced.


The tunica media of the embryonic aortic arch arteries, with the exception of the ductus arteriosus, is formed by migrating *cardiac neural crest cells*. These cells produce the *elastic mediae* specific to these vessels (see p. 314 .

Development of the Arteries


Apart from the aortae none of the main vessels of the adult arise as single trunks in the embryo. Along the course of each vessel a capillary network is first laid down and by selection and enlargement of definite paths in this the larger arteries and veins are defined. The branches of main arteries are not always simple modifications of the vessels of a capillary network but arise as outgrowths from the enlarged stem.

As mentioned, subsequent to head fold formation each primitive aorta consists of ventral and dorsal parts which are continuous through the first embryonic aortic arch. The dorsal aortae run caudally, one on each side of the notochord, but in the fourth week they fuse from about the level of the fourth thoracic to that of the fourth lumbar segment to form a single definitive descending aorta. Although in many animals paired ventral aortae arise from the truncus arteriosus and course headwards on the ventral surface of the pharynx, in the human embryo the ventral aortae are fused and form a dilated aortic sac (see **3.179A**  and consult Congdon 1922 ). The first aortic arches run through the mandibular arches, and caudal to them five additional pairs are developed within the corresponding pharyngeal arches so that in all six pairs of aortic arches are formed (**3.180A** ). The fifth arches are atypical and probably transient, at most, in mankind.

In fishes the aortic arches persist and give off branches to the gills, in which the blood is oxygenated. In mammals some of the arteries remain as permanent structures, while others disappear or are obliterated (**3.180** .







Caution should moderate unqualified use of the term *aortic arch(es)*. The *embryonic aortic arches* are paired bilateral series joining the ventral aortae (or their fused expanded homologue) with the dorsal aorta of its side after traversing the core of a pharyngeal arch. In contrast the *definitive aorta* consists of ascending aorta, aortic arch and descending (thoracic and abdominal) aorta—all parts of a single vessel in the mature state but derived from multiple embryonic sources. For one detailed analysis see **3.180** .

Aortic Sac

This represents the fused, paired ventral aortae (**3.179** ). As the embryo grows and the aorticopulmonary septum is formed, part of the caudal end of the sac is incorporated in the pulmonary trunk. The cranial end of the sac becomes drawn out into *right* and *left limbs* as the neck lengthens. The right limb becomes the brachiocephalic trunk and the left limb forms that part of the definitive arch of the aorta which lies between the origin of the brachiocephalic trunk and the left common carotid artery. The remainder of the sac contributes to the formation of the

ascending arch of the aorta.

Embryonic Aortic Arches

The embryonic aortic arches (3.180 ) , with the exception of the fifth, are developed in a craniocaudal sequence, but the more cranial are in process of disappearing before the caudal ones are completed. The first and second embryonic aortic arches are already dwindling by the time the third is established. The first disappears entirely. The dorsal end of the second arch or *hyoid artery* remains as the stem of the *stapedial artery*, while the remainder of this arch also disappears (3.181 ) . The *external carotid artery* first appears as a sprout which grows headward from the aortic sac close to the ventral end of the third arch artery. The *common carotid* arises from an elongation of the adjacent part of the aortic sac, and the third arch artery becomes the proximal part of the *internal carotid artery*. (Evidence against this view, however, has also been recorded: e.g. see Moffat 1959 ; Adams 1957 ). The fourth embryonic aortic arch on the right forms the proximal part of the *right subclavian artery*, whilst the corresponding vessel on the left is believed to constitute the *arch of the definitive aorta* between the origins of the *left common carotid* and *left subclavian arteries*. It has, however, proved difficult to assess accurately the contributions of the fourth embryonic aortic arches and it has also been variously claimed that the left fourth aortic arch is subsequently drawn into the descending or ascending (or both) limbs of the definitive aortic arch, and the corresponding vessel of the right contributes to the brachiocephalic artery. The identity and status of the fifth embryonic aortic arch artery is uncertain; it is usually incomplete and may connect the fourth aortic arch or subjacent aortic sac with the dorsal ends of the sixth aortic arch (whereas the other embryonic aortic arches pass between sac and dorsal aorta). The fifth aortic arch eventually disappears on both sides. From its inception the sixth embryonic arch vessel is associated with a developing lung bud. Initially each bud is supplied by a capillary plexus from the aortic sac. Later the plexus connects with the dorsal aorta and the sixth aortic arch is defined as a channel in the vascular connection between sac and dorsal aorta; however, this continues to supply the developing lung bud. When the aorticopulmonary septum divides the *truncus arteriosus* into pulmonary trunk and ascending aorta the sixth aortic arches retain continuity with the former. On the right the ventral part of the sixth aortic arch persists as the stem of the right pulmonary artery, but its dorsal segment disappears, possibly due to a decreased blood flow resulting from partitioning of the aortic and pulmonary bloodstreams (Navaratnam 1963 ). On the left side the ventral part of the sixth aortic arch is absorbed into the pulmonary trunk, while its dorsal segment persists as the *ductus arteriosus*, which is functional during intrauterine life but becomes obliterated after birth, ultimately forming the fibrous *ligamentum arteriosum*. Postnatal *functional* closure nears completion within a few weeks but *structural* changes continue over many months (p. 107 ).


The transformation of the aortic arches described above is conditioned by environmental changes and results largely from changes in the pharynx and from the descent of the heart. The whole period of transformation can be divided, both temporally and spatially, into two phases, pharyngeal and postpharyngeal. In the *pharyngeal phase*, which lasts until about the 12 mm CR length stage (stage 17), the arrangement of the aortic arches resembles that in lower vertebrates. In this phase the course of the blood from the heart to the dorsal aorta follows a succession of different pathways—first arch, first and second arches, second and third arches, third and fourth

aortic arches and finally third, fourth and sixth aortic arches. In the *postpharyngeal phase*, which extends onwards into, and beyond, intrauterine life the definitive human pattern and disposition of the vessels is finally established.


Tunica Media of the Embryonic Aortic Arches

In the early embryo, the tunica media of the third, fourth and sixth arches and the dorsal aorta contains smooth muscle cells that are not elastogenic and not of cardiac neural crest origin. The mediae of those vessels which do not receive a contribution from the neural crest, e.g. the intrapulmonary arteries or the subclavian arteries, continue to accumulate layers of smooth muscle derived from the surrounding mesenchyme. The embryonic aortic arch arteries, however, become surrounded by neural crest very early although there is initially no expression of either smooth muscle or elastin antigens by these cells. Later a larger population of crest cells migrates around these vessels and differentiates into an elastogenic phenotype. Neural crest cells differentiate in a downstream progression around the vessels, from the truncus arteriosus to the aortic arch arteries, until all of them are elastogenic. At the same time the original smooth muscle cells disappear along the great vessels to their first branch point (Rosenquist & Beall 1990^[4]). Ablation of the cardiac neural crest leads to changes in the embryonic aortic arch vessels: they may be absent, too large, too small, or aberrant in their connections and there is loss of bilateral symmetry. There is a significant decrease in the quantity of mesenchyme around these vessels resulting in direct apposition of endothelium and pharyngeal endodermal epithelium (Bockmann et al 1990^[4]). It should be noted that the *ductus arteriosus* (V1th aortic arch artery) and the *pulmonary arteries* have a *muscular tunica media* and not an elastic media as the other arch arteries. Thus just prior to, and after, birth the local action of prostaglandins can cause the ductus arteriosus to constrict and ultimately to close. *Coarctation of the aorta* is a condition in which the aorta is constricted, usually just above (preductal) or below (postductal) the entrance of the ductus arteriosus. An abnormal disposition of a smooth muscle media around the aorta at this point rather than an elastogenic media derived from the cardiac neural crest could result from an imperfect migration of crest cells into the aortic arch. The result would be an abnormal constriction of the aorta after birth.

Cranial Arteries

These develop in outline as follows (Padget 1948^[4]; 3.181 ). The *internal carotid artery* is progressively formed from the third arch artery, the dorsal aorta cranial to this and a further forward continuation which differentiates, at the time of regression of the first and second aortic arches, from the capillary plexus extending to the walls of the forebrain and midbrain. At its anterior extremity this *primitive internal carotid artery* divides into *cranial* and *caudal* divisions, the former terminating as the *primitive olfactory artery*, supplying the developing regions implied, and the latter sweeping caudally to reach the ventral aspect of the midbrain, its terminal branches being the *primitive mesencephalic arteries*. Simultaneously bilateral longitudinal channels differentiate along the ventral surface of the hindbrain from a plexus fed by intersegmental and transitory presegmental branches of the dorsal aorta and its forward continuation. The most important of the presegmental branches is closely related to the fifth nerve—the *primitive trigeminal artery*. *Otic* and *hypoglossal* presegmental arteries also occur

(Padget 1948^[4]); sometimes these persist. The longitudinal channels later connect, cranially, with the caudal divisions of the internal carotid arteries, each of which gives rise to an *anterior choroidal artery* supplying branches to diencephalon, including the telae choroideae, midbrain, and caudally with the vertebral arteries through the first cervical intersegmental arteries. Fusion of the longitudinal channels results in the formation of the *basilar artery*, whilst the caudal division of the internal carotid artery becomes the *posterior communicating artery* and the stem of the *posterior cerebral artery*. The remainder of the latter develops comparatively late, probably from the stem of the *posterior choroidal artery* which is annexed by the caudally expanding cerebral hemisphere, its distal portion becoming a *choroidal branch of the posterior cerebral artery*. Note that the posterior choroidal artery is supplying the tela choroidea at the future temporal end of the choroidal fissure, its rami advancing through the tela to become confluent with branches of the anterior choroidal artery (see above). In the rat, where the vascular pattern is essentially similar to that in man, this artery is derived from the posterior communicating artery, the common stem of origin of the posterior choroidal, mesencephalic and diencephalic arteries, together with a new channel formed in the plexus on the medial wall of the cerebral hemisphere initially supplied by the anterior choroidal artery (Moffat 1961a^[4]). The cranial division of the internal carotid artery gives rise to *anterior choroidal*, *middle cerebral* and *anterior cerebral arteries*, the stem of the primitive olfactory artery remaining as a small medial striate branch of the anterior cerebral artery. In the rat the primitive olfactory artery and its recurrent branch form the anterior cerebral artery, the territory of which is initially supplied by the primitive maxillary and the cranial ramus of the internal carotid arteries (Moffat 1961b^[4]). The *cerebellar arteries*, of which the superior is the first to differentiate, emerge from the capillary plexus on the wall of the rhombencephalon.

The source of the blood supply to the territory of the trigeminal nerve varies at different stages in development. When the first and second aortic arch arteries begin to regress, the supply to the corresponding arches is derived from a transient *ventral pharyngeal artery*, which grows from the aortic sac. It terminates by dividing into *mandibular* and *maxillary branches*. Later the *stapedial artery* develops from the dorsal stem of the second arch artery and passes through the condensed mesenchymal site of the future ring of the stapes to anastomose with the cranial end of the ventral pharyngeal artery thereby annexing its terminal distribution. The fully developed stapedial artery possesses three branches, *mandibular*, *maxillary* and *supraorbital*, which follow the divisions of the trigeminal nerve (3.181 ). The mandibular and maxillary branches diverge from a common stem. When the external carotid artery emerges from the base of the third arch it incorporates the stem of the ventral pharyngeal artery, and its maxillary branch communicates with the common trunk of origin of the maxillary and mandibular branches of the stapedial artery and annexes these vessels. The proximal part of the common trunk persists as the root of the *middle meningeal artery*. More distally the meningeal artery is derived from the proximal part of the supraorbital artery. The maxillary branch becomes the infraorbital artery and the mandibular branch forms the inferior alveolar artery.

When the definitive *ophthalmic artery* differentiates as a branch from the terminal part of the internal carotid artery, it communicates with the supraorbital branch of the stapedial artery; distally this becomes the *lacrimal artery*. The latter retains an anastomotic connection with the middle meningeal artery. The dorsal stem of the original second arch artery remains as one or

more *caroticotympanic branches* of the internal carotid artery.

At stage 20–23 (7–8 weeks), further expansion of the cerebral hemispheres produces the completion of the *circle of Willis*, with the development of the *anterior communicating arteries* by 8 weeks gestation. An annular network of meningeal arteries originates, mainly, from each middle cerebral artery and passes over each developing cerebral hemisphere; caudally similar meningeal branches arising from the *vertebral* and *basilar arteries* embrace the cerebellum and brainstem (Van den Bergh & Vander Eecken 1968^[1]). The further development of the telencephalon (see p. 247^[2]) somewhat obscures this early pattern over the cerebrum.

The meningeal arteries so formed have been classified into three groups, *paramedian*, *short circumferential* and *long circumferential arteries*. They can be described both supratentorially and infratentorially; all give off fine side branches and end as *penetrating arteries*. Of the supratentorial vessels, the paramedian arteries have a short course prior to penetrating the cerebral neuropil (e.g. branches of the *anterior cerebral artery*); the short circumferential arteries have a slightly longer course before becoming penetrating arteries (e.g. the *striate artery*); the long circumferential arteries reach the dorsal surface of the hemispheres. Infratentorial meningeal arteries are very variable. The paramedian arteries, after arising from the basilar or vertebral arteries, penetrate the brainstem directly; the short circumferential arteries end at the lateral surface of the brain before penetration; the long circumferential arteries later form the range of cerebellar arteries. Note that these vessels arranged as a series of loops over the brain arise from the circle of Willis and brainstem vessels on the base of the brain.

At 16 weeks gestation, the *anterior*, *middle* and *posterior cerebral arteries* contributing to the formation of the circle of Willis are well established. The meningeal arteries arising from them display a simple pattern with little tortuosity and very few branches. With the increasing age of the fetus and acquisition of the gyral pattern on the surface of the brain, their tortuosity, diameter and number of branches increase. This branching pattern is completed by 28 weeks gestation and the number of branches does not increase further (Takashima & Tanaka 1978^[3]). Numerous anastomoses (varying in size from 200–760 μm) occur between the meningeal arteries in the depths of the developing sulci, nearly always in the *cortical boundary zones* of the three main cerebral arteries supplying each hemisphere. The number, diameter and location of these anastomoses changes as fetal growth progresses due to a regression of the complex embryonic cerebral vascular system (see below). The boundary zones between the cerebral arteries may be the sites of inadequate perfusion in the premature infant.

Vascularization of the Brain

The brain becomes vascularized by angiogenesis (angiotrophic vasculogenesis; see p. 298^[4]) rather than by direct invasion by angioblasts. Blood vessels form by sprouting from vessels in the pial plexus, which surrounds the neural tube from an early stage (Noden & Li 1991^[5]). In the quail vascularization begins in the hindbrain on day 4 of incubation. Using antibodies against endothelial cells, endothelial sprouts were seen to penetrate the neuroepithelium on each side of the midline at inter-rhombomeric junctions (see p. 225^[6]; Noden & Li 1991^[5]). These sprouts

form branches which elongate at the junction between the ventricular and marginal zones; the branches project laterally within the inter-rhombomeric boundaries and longitudinally adjacent to the median floorplate. Subsequently, additional sprouts penetrate the inter-rhombomeric regions on the walls and floor of the hindbrain. Branches from the latter elongate towards and join the branches in the inter-rhombomeric junctions, forming primary vascular channels between rhombomeres and longitudinally on each side of the median floorplate (Noden 1991^[4]). Later additional sprouts invade the hindbrain within the rhombomeres, anastomosing in all directions.

The meningeal perforating branches pass into the brain parenchyma as *cortical*, *medullary* and *striate branches* (3.182^[5]). The cortical vessels supply the cortex via short branches which may form precapillary anastomoses, whereas the medullary branches supply the white matter. The latter converge towards the ventricle but rarely reach it; they often follow a tortuous course as they pass around nervous fascicles. The striate branches which penetrate into the brain through the anterior perforated substance (p. 1191^[6]), supply the basal nuclei and internal capsule via a sinuous course; they are larger than the medullary branches and the longest of them reach close to the ventricle. The periventricular region and basal nuclei are also supplied by branches from the *tela choroidea*; this develops from the early pial plexus but becomes medially and deeply placed as the telencephalon enlarges.

The cortical and medullary branches irrigate a series of cortico–subcortical cone-shaped areas, centred around a sulcus containing an artery. They supply a peripheral portion of the cerebrum and are grouped as *ventriculopetal arteries*. Striate branches, on the other hand, arborize close to the ventricle supplying a more central portion of the cerebrum; they, with branches from the tela choroidea, give rise to *ventriculofugal arteries*. The latter supply the ventricular zone (germinal matrix of the brain) and send branches towards the cortex. The ventriculopetal and ventriculofugal arteries run towards each other but they do not make any connections or anastomoses (3.182^[5]). The ventriculopetal vessels supply relatively more mature regions of the brain compared to the ventriculofugal, which are subject to constant remodelling and do not develop tunicae mediae until ventricular zone proliferation is completed. The *boundary zone* between these two systems (an outer centripetal and inner centrifugal) has practical implications related to the location of ischaemic lesions (periventricular leucomalacia, PVL) in the white matter of premature infant brains. The distribution of ischaemic lesions coincides with the demarcation zone between the centrifugal and centripetal vascular arterial systems (Wigglesworth & Pape 1978^[7]; also see below).

The same pattern of centripetal and centrifugal arteries develops around the fourth ventricle (Van den Bergh & Vander Eecken 1968^[8]). The ventriculofugal circulation is more extensive in the cerebellum than in the telencephalon. The arteries arise from the various cerebellar arteries and course, with the cerebellar peduncles, directly to the centre of the cerebellum by-passing the cortex. The ventriculopetal arteries derive from the meningeal vessels over the cerebellar surface and most terminate in the white substance.

At 24 weeks of gestation, there is a relatively well developed blood supply to the basal nuclei and internal capsule, through a prominent *Heubner's artery* (*arteria recurrens anterior*, see p.

1528), a branch of the anterior cerebral artery. The cortex and the white matter regions are rather poorly vascularized at this stage. Injection studies have demonstrated the distribution of arteries and veins on the lateral aspect of the cerebral hemispheres and shown that it is naturally affected by the formation of the Sylvian (lateral cerebral) fissure and development of cerebral sulci and gyri (Okudera et al 1988). Between 12 and 20 weeks gestation the middle cerebral artery and its branches are relatively straight with branching in an open-fan pattern. At the end of 20 weeks, the arteries become more curved as the opercula begin to appear and submerge the insular cortex. The area supplied by the middle cerebral artery becomes predominant when compared to the territories supplied by the anterior and posterior cerebral arteries. Early arterial anastomoses appear around 16 weeks gestation and increase in size with advancing age. The sites of anastomoses between the middle and anterior cerebral arteries move from the convexity of the brain towards the superior sagittal sinus. Anastomotic connections between the middle and posterior cerebral arteries shift towards the basal aspect of the brain.

By 32–34 weeks, marked involution of the ventricular zone (germinal matrix) takes place and the cortex acquires its complex gyral pattern and, associated with it, an increased vascular supply. The ventricular zone capillaries are gradually remodelled to blend with the capillaries of the caudate nucleus. Heubner's artery eventually supplies only a small area at the medial aspect of the head of the caudate nucleus. In the cortex, there is progressive elaboration of the cortical blood vessels (3.182), and towards the end of the third trimester, the balance of cerebral circulation shifts from a central, basal-nuclei oriented circulation to a circulation predominant in the cortex and the white matter (3.182). These changes in the pattern of cerebral circulation are of major significance in pathogenesis and distribution of hypoxic/ischaemic lesions in the developing human brain. In a *premature brain*, the majority of ischaemic lesions occur in the boundary zone between the centripetal and centrifugal arteries, in the periventricular white matter. In the *full-term infant* the cortical boundary zones and watershed areas between different arterial blood supplies are similar to those in adults.

Vessels of the Ventricular Zone (Germinal Matrix)

It has been suggested that the germinal matrix (the region of the developing brain termed ventricular zone by the Bolder Committee, see p. 252), but often referred to clinically, somewhat imprecisely, as the germinal matrix) is particularly prone to ischaemic injury in the immature infant because of its unusual vascular architecture. A microangiographic study of the structure of the vessels in the periventricular matrix (Takashima & Tanaka 1978) established that the germinal matrix is the end zone or border zone between the *cerebral arteries* and the *collection zone* of the *deep cerebral veins*. The subependymal veins (*septal*, *choroidal*, *thalamostriate* and *posterior terminal*, see p. 1204) flow **towards** the interventricular foramen with a sudden change of flow at the level of the foramen, where the veins recurve at an acute angle to form the paired *internal cerebral veins*. The capillary channels in the germinal matrix open at right angles, directly into the veins (Hambleton & Wigglesworth 1976). It has been postulated that these small vessels may be points of vascular rupture and the site of subependymal haemorrhage.

The capillary bed in the ventricular zone is supplied mainly by Heubner's artery and terminal

branches of the lateral striate arteries from the middle cerebral artery (Wigglesworth 1980^[4]). As the highly cellular structure of the ventricular zone is a temporary feature, the vascular supply to this area displays some primitive features and has the capacity to remodel when, towards the end of gestation, the ventricular zone cells migrate and the remaining cells differentiate as ependyme (Wigglesworth & Pape 1980^[4]).

Some studies have shown that vessel density is relatively low in the ventricular zone and that this area may normally have a relatively low blood flow (Pasternak et al 1982^[4]). Immature vessels, without a complex basal lamina or glial sheet, have been described up to 26 weeks gestation in the zone (Larroche 1982^[4]), and it has been reported that the endothelium of these vessels is thinner than in the cortical vessels (Trommer et al 1987^[4]). In infants of less than 30 weeks gestation, the vessels in the ventricular zone contain no smooth muscle, collagen or elastic fibres (Haruda & Blanc 1981^[4]). Collagen and smooth muscle were seen in other regions after 30 weeks but were not detected in the remains of the zone (germinal matrix). The lack of these components could make the vessels in the ventricular zone vulnerable to changes in the intraluminal pressure and the lack of smooth muscle would debar them from participating in autoregulatory processes.

Hegedus and Molnar (1985^[4]) have shown that cerebral vessels in premature infants lack elastic fibres and have a disproportionately small amount of reticulin fibres. An electron microscopic study of the cortical and germinal plate blood vessels showed that in infants of between 25 and 32 weeks gestation the germinal matrix vessels consisted commonly of 1–2 endothelial cells with an occasional pericyte, and the capillary lumina were larger than those of the vessels in the cortex. In more mature infants the basal lamina was thicker and more irregular when compared to cortical vessels (Grunnet 1989^[4]).


Glial fibrillary acidic protein (GFAP) positive cells have been detected around blood vessels in the germinal matrix from 23 weeks gestation (Gould & Howard 1988^[4]). Glial cells may contribute to changes in the nature of endothelial intercellular junctions in brain capillaries (Tae-Cheng et al 1986^[4]).

Dorsal Aortae

These persist on the cranial side of the third aortic arches as continuations of the internal carotid arteries (**3.88A-C**^[4]). The dorsal aorta between the third and fourth aortic arches, the *ductus caroticus*, diminishes and finally disappears; but from fourth arch to the origin of the seventh intersegmental artery the right dorsal aorta becomes part of the right subclavian artery (**3.177**^[4]). Caudal to the seventh intersegmental artery the right dorsal aorta disappears as far as the locus of fusion of thoracic aortae. After disappearance of the left ductus caroticus, the remainder persists to form the descending part of the arch of the aorta. Thence the fused right and left embryonic dorsal aortae persist as the definitive descending thoracic and abdominal aorta. A constriction, the *aortic isthmus*, is sometimes present in the aorta between the final site of origin of the left subclavian artery and reception of the ductus arteriosus (see *coarctation* of the aorta, p. 1510^[4]).

In the adult, the right subclavian artery occasionally arises from the arch of the aorta distal to the origin of the left subclavian and then passes upwards and to the right behind the trachea and oesophagus. This condition is possibly explained by the persistence of the embryonic right dorsal aorta and the obliteration of the fourth aortic arch of the right side.



In birds the right fourth aortic arch is transformed into the definitive arch of the aorta; in reptiles the fourth arches of both sides persist and give rise to their characteristic double aortic arch. In both these classes, development of the heart and aortic arches is probably along phylogenetic lines so divergent from the mammalian pattern that comparisons may be inappropriate.

The heart originally lies ventral to the pharynx, immediately caudal to the stomodeum (3.179A ); with the elongation of the neck and the development of the lungs it recedes within the thorax and, correspondingly, the vessels are drawn out and the original position of the fourth and sixth aortic arches is greatly modified. Thus, on the right the fourth aortic arch only recedes to the thoracic inlet, while on the left side it descends **into** the thorax. The recurrent laryngeal nerves (in contrast to the other arch nerves) originally pass to the larynx **caudal** to the sixth pair of aortic arches, and are therefore affected by the descent of these structures; thus in the adult the left nerve hooks round the ligamentum arteriosum **within** the thorax; on the right owing to the disappearance of the fifth and the dorsal part of the sixth aortic arch, the right recurrent laryngeal nerve hooks round the fourth aortic arch, i.e. the commencement of the right subclavian artery (i.e. just **above** the thoracic inlet).

At first the aortae are the only longitudinal vessels present, for their branches all run at right angles to the long axis of the embryo. Later these transverse arteries become connected in certain situations by longitudinal anastomosing channels, which in part persist, forming such arteries as the internal thoracic, the superior and inferior epigastric, the gastro-epiploic, etc. Each primitive dorsal aorta gives off:

- ventral splanchnic arteries, paired segmental branches to the digestive tube
- lateral splanchnic arteries, paired segmental branches to the mesonephric ridge
- somatic arteries, intersegmental branches to the body wall.

Ventral Splanchnic Arteries

Originally paired vessels, these are distributed to the capillary plexus in the wall of the yolk sac. After fusion of the dorsal aortae they merge as unpaired trunks distributed to the increasingly defined and lengthening primitive digestive tube. Longitudinal anastomotic channels connect these branches along the dorsal and ventral aspects of the tube, forming dorsal and ventral splanchnic anastomoses (3.183 ; Ennablie & Niveiro 1967 ). These vessels obviate the need for so many 'subdiaphragmatic' ventral splanchnic arteries, and these are reduced to three: the coeliac trunk and superior and inferior mesenteric arteries. As the viscera supplied descend into the abdomen their origins migrate caudally by differential growth; thus the origin of the coeliac artery is transferred from the level of the seventh cervical segment to the level of the twelfth

thoracic, the superior mesenteric from the second thoracic to the first lumbar and the inferior mesenteric from the twelfth thoracic to the third lumbar. (Above the diaphragm, however, a variable number of ventral splanchnic arteries persist, usually four or five, supplying the thoracic oesophagus.) The dorsal splanchnic anastomosis persists in the gastro-epiploic, pancreaticoduodenal and the primary branches of the colic arteries, while the ventral splanchnic anastomosis forms the right and left gastric and the hepatic arteries. These arterial rearrangements have been investigated recently by angiography and explanatory haemodynamic hypotheses have been advanced (Barth et al 1976^[4]).

Lateral Splanchnic Arteries

These supply, on each side, the mesonephros, metanephros, the testis or ovary, and the suprarenal gland; all these structures develop, in whole or in part, from the intermediate mesenchyme of the mesonephric ridge (p. 200^[4]). One testicular or ovarian artery and three suprarenal arteries persist on each side. The phrenic artery branches from the most cranial suprarenal artery, and the renal artery arises from the most caudal. Additional renal arteries are frequently present and may be looked on as branches of persistent lateral splanchnic arteries.

Somatic Arteries

Intersegmental in position, they persist, almost unchanged, in the thoracic and lumbar regions, as the posterior intercostal, subcostal and lumbar arteries. Each gives off a dorsal ramus which passes backwards in the intersegmental interval and divides into medial and lateral branches to supply the muscles and superficial tissues of the back (3.183^[4]). It also gives off a spinal branch, which enters the vertebral canal and divides into a series of branches to the tissues constituting the walls and joints of the osteoligamentous canal and neural branches to the spinal cord and spinal nerve roots (Somogyi et al 1973^[4]; Undi et al 1973^[4]). Having produced its dorsal branch the intersegmental artery runs ventrally in the body wall, gives off a lateral branch and terminates in muscular and cutaneous rami. Before their division, the stems of the somatic arteries, at thoracic and lumbar levels, provide small rami which enter the developing vertebral bodies.

Numerous longitudinal anastomoses link up the intersegmental arteries and their branches (3.183^[4]). On both sides a *postcostal anastomosis* connects their dorsal branches in the intervals between the necks of ribs and the vertebral transverse processes. This persists in the cervical region where it forms the greater part of the vertebral artery. A *post-transverse anastomosis* also connects the dorsal branches and forms the greater part of the deep cervical artery. A *precostal anastomosis* connects intersegmental arteries beyond the origins of their dorsal branches. The ascending cervical and the superior intercostal arteries are persistent parts of this vessel. Lastly, near the anterior median line intersegmental arteries are linked by a *ventral somatic anastomosis*. Most of these vessels persist bilaterally as the internal thoracic, the superior and inferior epigastric arteries.

Umbilical arteries at first are the direct caudal continuation of the primitive dorsal aortae and are



present in the body stalk before any vitelline (yolk sac) or visceral branches emerge, indicating the dominance of the allantoic over the vitelline circulation in the human embryo. (On a comparative basis the umbilical vessels are chorio-allantoic and therefore 'somatovisceral'.) After the fusion of the dorsal aortae the umbilical arteries arise from their ventrolateral aspects and pass medial to the primary excretory duct (Wolffian) to the umbilicus. Later the proximal part of each umbilical artery is joined by a new vessel which leaves the aorta at its termination and passes lateral to the primary excretory duct. This, possibly the fifth lumbar intersegmental artery, constitutes the *dorsal root* of the umbilical artery (the original stem, the *ventral root*). The dorsal root gives off the axial artery of the lower limb, branches to the pelvic viscera and, more proximally, the external iliac artery. The ventral root disappears entirely, the umbilical artery now arising from that part of its dorsal root distal to the external iliac artery, i.e. the internal iliac artery.




Arteries of the Limbs




The early limb bud receives blood via intersegmental arteries which contribute to a primitive capillary plexus. At the tip of the limb bud there is a *terminal plexus* that is constantly renewed in a distal direction as the limb grows. Later one main vessel supplies the limb and the terminal plexus; it is termed the *axial artery*. The terminal plexus is separated from the outer ectodermal sleeve of the limb by an *avascular zone* of mesenchyme. Experiments in which portions of ectoderm are implanted into a part of the limb that would otherwise be vascularized result in an avascular zone forming around the ectoderm. The avascular region contains an extracellular matrix consisting largely of hyaluronic acid. Removal of this hyaluronic acid by hyaluronidase results in vascularization of the tissue since partial degradation products of hyaluronic acid are angiogenic (Feinberg 1991¹). Thus ectodermal/mesenchymal interactions and extracellular matrix components are controlling the initial patterning of blood vessels within the limb. During these early stages of limb development the proximodistal regions of the limb are patterned by the *progress zone* beneath the *apical ectodermal ridge* (see p. 288²), later after the main elements of the limb have developed the ectoderm and underlying mesenchyme interact to produce the epidermis and dermis (see p. 294³). It may be that the early presence of the avascular zone ensures little interaction of these tissues until a later stage of development, thus preventing premature skin development.

The development of the vasculature in the limb precedes the morphological and molecular changes that occur within the limb mesenchyme. The differentiation of cartilage within the limb occurs only after local vascular regression begins, and only in areas with few or no capillaries. Regions of mesenchyme free of capillaries and at high cell density are the sites of chondrogenesis (see p. 291⁴). Whether the presence, or lack, of blood vessels, by varying the supply of nutrients to the tissue can confer different local environmental stimuli for mesenchymal cells, thus resulting in heterogeneous differentiation of the tissue of the limb, or whether, on the other hand, the local environment is controlled by the diversity of the endothelial cells is not known. Similarly, it is not clear whether inductive factors from the limb mesenchyme cause the changes in the blood vessel pattern.

In the *upper limb bud*, usually only one trunk, the subclavian, persists and it probably represents

the lateral branch of the *seventh intersegmental artery*. Its main continuation (*axis artery*) to the upper limb (3.184 ) , later the *axillary* and *brachial arteries*, passes into the forearm deep to the flexor muscle mass and terminates as a deep plexus in the developing hand. This vessel ultimately persists as the *anterior interosseous artery* and the *deep palmar arch*. A branch from the main trunk passes dorsally between the early radius and ulna as the *posterior interosseous artery*, while a second accompanies the median nerve into the hand, where it ends in a *superficial capillary plexus*. The *radial* and *ulnar arteries* are the latest arteries to appear in the forearm; at first the radial artery arises more proximally than the ulnar and crosses in front of the median nerve, supplying the biceps. Later, the radial artery establishes a new connection with the main trunk at or near the level of origin of the ulnar artery and the upper portion of its original stem usually disappears to a large extent (see also p. 1540 ) . On reaching the hand the ulnar artery becomes linked up with the superficial palmar plexus, from which the *superficial palmar arch* is derived, while the median artery commonly loses its distal connections and is reduced to a small vessel. The radial artery passes to the dorsal surface of the hand but, after giving off dorsal digital branches, it traverses the first intermetacarpal space and joins the deep palmar arch.

Because of their multiple and plexiform sources, the temporal succession of emergence of principal arteries, anastomoses and periarticular networks and functional dominance followed by regression of some paths, anomalies of the forelimb arterial tree are fairly common. In the main, such anomalous patterns present as: divergences in the mode and proximodistal level of branching; the presence of unusual compound arterial segments; aberrant vessels connecting other principal vessels, arcades or plexuses; and vessels occupying exceptional tissue planes (e.g. superficial fascia instead of the usual subfascial route) or having unexpected neural, myological or osteoligamentous relationships. For example, see variations on pages 1539 , 1541 , 1543 .

The *axial artery of the lower limb* (3.185 ) arises from the dorsal root of the umbilical artery, and courses along the **dorsal** surface of the thigh, knee and leg; below the knee it lies between tibia and popliteus and in the leg between the crural interosseous membrane and tibialis posterior. Ending distally in a *plantar network*, it gives off a perforating artery traversing the sinus tarsi to form a *dorsal network*. The *femoral artery* passes along the **ventral** surface of the thigh, opening a new channel to the lower limb. It arises from a capillary plexus, connected proximally with the femoral branches of the external iliac artery and distally with the axis artery. At the proximal margin of the popliteus the axis artery provides a *primitive posterior tibial* and a *primitive peroneal branch*, which run distally on the dorsal surface of that muscle and on tibialis posterior to gain the sole of the foot. At the distal border of popliteus the axis artery gives off a *perforating branch*, which passes ventrally between the tibia and the fibula and then courses to the dorsum of the foot, forming the *anterior tibial artery* and *arteria dorsalis pedis*. The primitive peroneal artery communicates with the axis artery at the distal border of the popliteus and in its course in the leg (Senior 1919 , 1920 ) .

The femoral artery gradually increases in size and coincidentally most of the axis artery disappears; proximal to its communication with the femoral the root of the axis artery, however,

persists as the *inferior gluteal artery* and the *arteria comitans nervi ischiadici*.

The proximal parts of the primitive posterior tibial and peroneal arteries fuse, but distally remain separate. Ultimately much of the primitive peroneal artery disappears, although a part of the axis artery is incorporated in the permanent peroneal artery. As in the forelimb (above) the same considerations apply to anomalies and variations.

Development of the Veins

Often, for convenience and apparent simplicity, the early embryonic veins are segregated into two groups, *visceral* and *somatic*. The visceral group comprises the derivatives of the vitelline and umbilical veins; the somatic group includes all remaining veins. Such a classification is a potentially misleading oversimplification, based on inadequate criteria. Many embryonic veins, with time, change the principal tissues they drain; others have some radicles from patently parietal tissues that become confluent with drainage channels that are clearly visceral, thus forming a compound vessel; finally some veins differentiate from contrasting mesenchymal layers at different points along their course (each having its distinct phylogenetic history).

Less confusion attaches to the recognition of three main groups of veins—the *vitelline*, *umbilical* and *cardinal* vein complexes; nevertheless, even here, some imprecision persists where, for instance, anastomoses or convergence of radicles from different groups occurs, where ontogenetically compound tissues are drained or when distinct developmental layers are traversed. The early embryonic veins develop initially with a symmetric bilateral array of channels; the **principal** events correlated with changes in this are the craniocaudal and mediolateral gradients in growth and differentiation of the nervous system, skeleton and musculature; diversion of cardiac venous return to the right with concomitant cardiac asymmetry; 'descent' of the heart and lungs; gut rotation and repositioning; and venous involvement by the developing liver, pancreas, spleen and mesonephric ridges. (Clearly, the temporospatial development sequences of **all** vascularized tissues are involved to varying, but less obvious, extents.) As noted, the primitive tubular symmetric heart receives its venous return through the right and left sinusal horns (horns of the sinus venous). The horns are initially embedded in the mesenchyme of the septum transversum and each receives, most medially, the termination of the principal vitelline vein (but see below), more laterally, the umbilical vein and, most laterally, having encircled the pleuroperitoneal canal, the common cardinal vein. These cardiac inputs correspond, in large measure but not exclusively, with the groups of veins mentioned above.



Vitelline Veins

The vitelline veins drain capillary plexuses developed in the splanchnopleuric mesenchyme of the secondary yolk sac. With head, tail and lateral fold formation, the upper recesses of the yolk sac are enclosed within the embryo as the splanchnopleuric gut tube extending from the stomodeal buccopharyngeal membrane to the proctodeal cloacal membrane. It may be

emphasized that derivatives from **all** these levels possess a venous drainage, originally **vitelline in origin**, although many accounts are limited to the (mainly subdiaphragmatic–sacral) regions drained via the hepatic portal vein. The deep aspects of the maxillomandibular facial prominences, retrogingival oral cavity, the pharyngeal walls and their lymphoid and endocrine derivatives and the cervicothoracic oesophagus, all have drainage channels that connect with the *precardinal complex*, ultimately returning blood to the heart via the superior vena cava. Laryngeal and tracheobronchial veins also drain to the precardinal complex, whilst the capillary plexuses developed in the (splanchnopleuric) walls of the fine terminal respiratory passages and alveoli, converge on *pulmonary veins* of increasing calibre, finally making secondary connections with the left atrium of the heart and may be grouped with the vitelline systems. Even the heart, itself, first differentiates in splanchnopleure that, after head fold formation, forms the dorsal wall of the primitive pericardial cavity (floor of the rostral foregut) and may therefore be considered a highly specialized vitelline vascular derivative. Similarly at the caudal extremity of the splanchnopleuric gut tube (the future lower rectum and upper anal canal; p. 191📖) the vitelline venous drainage makes connections with the internal iliac radicles of the *postcardinal complex*.


The increasingly extensive remainder of the gut tube, from the gastric terminal segment of the future oesophagus to the upper rectum, is, as elsewhere, clothed with splanchnopleuric mesenchyme permeated by a capillary plexus; the latter drains into an anastomosing network of veins. The net is denser ventrally and in the central midgut region; for a while, it receives a leash of small veins from the definitive yolk sac that enter the embryo through the umbilicus, embedded in the yolk stalk. Later, in normal development, both stalk and vessels atrophy. Within the splanchnopleuric net, progressing rostrally, longitudinal channels anterolateral to the gut become increasingly well defined—the embryonic abdominal *vitelline veins*. Entering the septum transversum, the right and left vitelline veins incline slightly, becoming parallel to the lateral aspects of the gut; they establish connections with capillary plexuses in the septal mesenchyme, then continue, finally curving to enter the medial part of the cardiac sinusal horn of their corresponding side. The parts of the gut closely related to the presinual segments of the vitelline veins, just described, are the future subdiaphragmatic end of the oesophagus, primitive stomach, the superior (first) and descending (second) regions of the duodenum, and the remainder of the duodenal tube. The early septum transversum is a thick mass of mesenchyme filling the interval between the median foregut and ventral body wall, and extending from the primitive pericardium to the rostral lip of the umbilicus; in it somatopleuric and splanchnopleuric mesenchymes are confluent. When, later, the cardiac sinuatrium rises into the pericardium, the rostral part of the septum (*pars diaphragmatica*) is one of the multiple contributors to the framework of the definitive diaphragm; its caudal *pars mesenterica* provides ventral mesenteries, serous coats and mural tissues for the abdominal foregut, listed above. Where foregut continues into midgut (initially the rostral rim of the yolk stalk) the hepatic (and, transiently, ventral pancreatic) rudiments form as protrusions of the gut; the rapid and asymmetric invasion of the septum cranioventrally is correlated with profound modifications of the transeptal parts of the vitelline and umbilical veins, and the splanchnopleuric mesothelium, framework and mesenteries of the liver, stomach and duodenum (pp. 192–198📖). The stem of the earliest hepatic rudiment projects from the ventral wall of the future second (descending) part of the duodenum; whilst expansion of the body of the liver continues as indicated, with gut rotation and differential growth, the ultimate derivative of the stem, the common bile duct, curves posterior to the

proximal duodenum to reach its termination through the left posteromedial aspect of the second part of the duodenum.





The principal ascending vitelline veins flanking the sides of the abdominal part of the foregut receive venules from its splanchnopleuric capillaries, and those of the septal mesenchyme. Within these venular nets, enlarged (but still plexiform) anastomoses connect the two vitelline veins. (For clarity these are represented diagrammatically as simple transverse channels, **3.186** ) A *subdiaphragmatic intervittelline anastomosis* develops in the rostral septal mesenchyme, lying a little caudal to the cardiac sinuatrial chamber, connecting the veins near their sinuatrial terminations. (Sometimes termed *suprahepatic* because of the position of the hepatic primordium; with expansion of the latter the channel becomes partly *intrahepatic*.) The presumptive duodenum is crossed by three transverse *duodenal intervittelline anastomoses*; their relation to the gut tube alternates: the most cranial, the *subhepatic*, is ventral, the *intermediate* is dorsal and the *caudal* is ventral. It has become customary to describe the paraduodenal vitelline veins and their associated anastomoses as forming a figure 8. At this early stage when left and right embryonic veins are still symmetric, the cranial duodenal anastomosis becomes connected with the subdiaphragmatic anastomosis by a *median* longitudinal channel, the *primitive ductus venosus*, which is dorsal to the expanding hepatic primordium, but ventral to the gut. The further development of the vitelline veins and anastomoses is, as indicated, closely interlocked with rapid hepatic expansion and gut changes; also umbilical vein disposition and modification is closely involved, and their early emergence and arrangement must be outlined before an account of later asymmetries is undertaken. The latter differs in part from long held descriptions, following the detailed analyses of goat and human embryos by Dickson (1957 .




Umbilical Veins

The umbilical veins form by the convergence of venules draining the splanchnopleure of the extraembryonic allantois. Throughout *Amniota* the allantois arises as a diverticulum from the caudal yolk sac wall (later, the ventrorostral wall of the cloacal part of the hindgut, or future bladder). Its degree of vesicular, then sometimes saccular, expansion is extremely variable; on occasion it virtually fills the extraembryonic coelom; the human endodermal allantois is diminutive, projecting merely into the embryonic end of the connecting stalk. The latter is regarded by many as precociously formed allantoic mesenchyme, and the umbilical vessels as *allantoic* (with close affinities, because of their common phylogenetic history, with the vitelline vessels). In the human embryo the peripheral venules drain the mesenchymal cores of the chorionic villous stems and terminal villi (extraembryonic *somatopleuric* structures); these are the radicles of a, usually single, *vena umbilicalis impar* which traverses the compacting mixed mesenchyme of the umbilical cord to reach the caudal rim of the umbilicus. Here, the single cordal vein divides into primitive right and left umbilical veins; each curves rostrally in the somatopleuric lateral border of the umbilicus (i.e. where intraembryonic and extraembryonic or amniotic somatopleure are continuous), where it lies **lateral** to the communication between both the intraembryonic and extraembryonic coeloms. Rostrolateral to the umbilicus the two umbilical veins reach, enter and traverse the junctional mesenchyme of the septum transversum, connect with septal capillary plexuses, then continue, to enter their corresponding cardiac sinuatrial horns (lateral to the terminations of the vitelline veins). This early symmetric disposition of the

vitelline veins and anastomoses, umbilical and common cardinal veins and locus of the hepatic primordial complex is summarized in 3.186 .

Changes in the Vitello-Umbilical Veins

Progressive changes in the vitello-umbilical veins are rapid, profound and closely linked with regional modifications of shape and position of the gut, expansion and invasion of venous channels by hepatic tissue, asymmetry of the heart and cardiac venous return. The principal events are summarized in 3.186 , 187 , 188  (see Dickson 1957  for details and bibliography).

From the pars hepatica of the ventral hepatopancreatic rudiment (p. 187 ) interconnected sheets and 'cords' of endodermal cells, the presumptive hepatocytes, penetrate the mesenchyme-filled spaces of the pre-existing septal capillary plexuses. Possibly, under the influence of the endodermal sheets, the plexuses become more profuse by the addition of angioblastic septal mesenchyme, which also forms masses of perivascular intrahepatic haemopoietic tissue. These processes extend along the plexiform connections of the vitelline, and later the umbilical veins until their intrahepatic (transeptal) zones themselves become largely plexiform—initially capillary in nature, but transforming into a mass of rather wider, irregular, sinusoidal vessels with a discontinuous endothelium containing many phagocytic cells (pp. 1802–1806 ). The lengths of vitelline veins involved in these processes are the intermediate parts of the segments extending from the subhepatic (cranioventral duodenal) to the suprahepatic (subdiaphragmatic) transverse intervittelline anastomoses and the corresponding lengths of the umbilical veins. Thus at this early stage the liver sinusoids are perfused by mixed blood reaching them through a series of branching vessels collectively called the *venae advehentes*, or *venae afferentes hepatis*; they are deoxygenated from the gut splanchnopleure via vitelline vein hepatic terminals and oxygenated from the placenta via hepatic terminals of the umbilical veins. Blood leaves the liver through four *venae revehentes* (*venae efferentes hepatis*): two on each side reach and open into their respective cardiac sinusal horns. This full complement of four *hepatocardiac veins* is only transient, becoming reduced to one dominant, rapidly enlarging channel. As detailed below, the originally bilaterally symmetric cardinal vein complexes, both rostral and caudal, develop transverse or oblique anastomoses whereby the cardiac venous return is restricted to the definitive right atrium. (The pulmonary veins are the only major ones returning to the left atrium.) The increasingly deep inflexion of the left wall of the common sinuatrial chamber, separation of the left sinusal horn and 'body' of the sinus venosus, movement to the right of the sinuatrial orifice, and right atrial inclusion of the right sinusal horn have been noted (p. 293 .

These cardiac and concomitant hepato-enteric changes are accompanied by events in supra-, intra- and subhepatic parts of the vitello-umbilical veins. Some vessels enlarge, persisting as definitive vessels to maturity and, in places, are joined later by other channels becoming defined in already established capillary plexuses. Other vessels retrogress, either disappearing completely or remaining as vestigial tags and occasionally vessels of fine calibre. Finally, some vessels of crucial importance in the circulatory patterns of embryonic and fetal life become obliterated **postnatally** and transformed to substantial fibrous cords. Both right and left



umbilical hepatocardiac and the left vitelline hepatocardiac veins continue, for a time, to discharge blood into their sinusal horns; however they begin to retrogress (3.186, 187). The right umbilical channel atrophies completely; the left channels also disappear, but their cardiac terminals may, on occasion, be found as conical fibrous tags attached to the inferior wall of the coronary sinus. The right vitelline hepatocardiac vein continues enlarging and ultimately forms the terminal segment of the inferior vena cava. The latter receives the right venae revehentes and new channels draining the territories of the left venae revehentes; these collectively form the upper and lower groups of right and (secondary) left hepatic veins. The terminal caval segment also shows the orifice of the right half of the intervittelline subdiaphragmatic anastomosis (p. 321) and a large new connection with the right subcardinal vein (see below).

The hepatic terminals of the right and left duodenal parts of the vitelline veins are destined to form the corresponding branches of the *hepatic portal vein*, the left branch incorporating the cranial ventral intervittelline anastomosis. With rotation of the gut and formation of the duodenal loop, segments of the original vitelline veins and the caudal transverse anastomosis (indicated in 3.186, 187, 188) atrophy, whilst new splanchnopleuric venous channels, the superior mesenteric and splenic veins, converge and join the left end of the dorsal intermediate anastomosis. The numerous other radicles of the portal vein and its principal branches, including the inferior mesenteric vein, are later formations.

For a period placental blood returns from the umbilicus via right and left umbilical veins, both discharging through venae advehentes into the hepatic sinusoids, where admixture with vitelline blood occurs. At approximately 7 mm CR length, the right umbilical vein retrogresses completely; the left umbilical vein retains some vessels discharging directly into the sinusoids, but new enlarging connections with the left half of the subhepatic intervittelline anastomosis emerge. The latter is the commencement of a *by-pass channel* for the majority of the placental blood, *which continues through the median ductus venosus* and finally the right half of the subdiaphragmatic anastomosis, to reach the termination of the inferior vena cava. Postnatally these channels are obliterated, with the resulting ligamentum teres extending from the umbilicus to the porta hepatis, whence, having established connections with the left branch of the portal vein, it continues as the ligamentum venosum to join an upper left hepatic vein, and terminates in the suprahepatic inferior vena cava.

Cardinal Venous Complexes

Cardinal venous complexes (3.189) are first represented by two large vessels on each side, the *precardinal* and *postcardinal veins*; the former drain the rostral part of the embryo, the latter its caudal region. The two veins on each side unite to form a short *common cardinal vein*, which passes ventrally, lateral to the pleuropericardial canal (p. 180), to open into the corresponding horn of the sinus venosus (3.179B). (The cardinal complexes are often, less appropriately, called parietal or somatic. In addition to drainage of the latter, they receive many radicles from splanchnopleuric structures.)

Owing to the rapid development of the head and brain the precardinal veins become enlarged. They are further augmented by the *subclavian* veins from the upper limb buds, and so become the chief tributaries of the common cardinal veins; these gradually assume an almost vertical position in association with the descent of the heart into the thorax. That part of the original precardinal vein rostral to the subclavian is now the *internal jugular vein*, and their confluence the *brachiocephalic vein* of each side. The right and left common cardinal veins are originally of the same diameter. By the development of a large oblique transverse connection, the *left brachiocephalic vein* carries blood across from the left to the right (3.179 ). The part of the original right precardinal vein between the junction of the two brachiocephalics and the azygos veins forms the upper part of the *superior vena cava*; the caudal part of the latter vessel (below the entrance of the azygos vein) is formed by the right common cardinal. Caudal to the transverse branching of the left brachiocephalic vein the left precardinal and left common cardinal veins largely atrophy, the former constituting the terminal part of the left superior intercostal vein, while the latter is represented by the ligament of the left vena cava and the oblique vein of the left atrium (3.189B ). The remainder of the left superior intercostal vein is developed from the cranial end of the postcardinal vein and drains the second, third and, on occasion, the fourth intercostal veins. The oblique vein passes downwards across the back of the left atrium to open into the coronary sinus, which, as already indicated, represents the persistent left horn of the sinus venosus. Right and left superior venae cavae are present in some animals, and occasionally persist in mankind.

Inferior Vena Cava


(3.189)

The inferior vena cava of the adult is a composite vessel, and the precise mode of development of its postrenal segment (caudal to the renal vein) is still somewhat uncertain. Its function is initially carried out by the right and left postcardinal veins, which receive the venous drainage of the lower limb buds and pelvis and run in the dorsal part of the mesonephric ridges, receiving tributaries from the body wall (*intersegmental veins*) and from the derivatives of the mesonephroi.


A second pair of longitudinal channels, the *subcardinal veins*, form in ventromedial parts of the mesonephric ridges and become connected to the postcardinal veins by a number of vessels traversing the medial part of the ridges. The subcardinal veins intercommunicate by a *pre-aortic anastomotic plexus*, which later constitutes the part of the *left renal vein* crossing anterior to the abdominal aorta.

The formation of an oblique transverse anastomosis between the iliac veins—which itself becomes the major part of the definitive *left common iliac vein*—diverts an increasing volume of blood into the right longitudinal veins, accounting for the ultimate disappearance of most of those on the left.

At its cranial end the subcardinal vein receives the *suprarenal vein* on each side, but on the right side it comes into intimate relationship with the liver. An extension of the vessel takes place in a cranial direction and meets and establishes continuity with a corresponding new formation which is growing caudally from the right vitelline hepatocardiac (common hepatic) vein. In this way on the right side a more direct route is established to the heart and the *prerenal* (cranial) segment of the inferior vena cava is defined.

The enlargement of the metanephros (p. 200 ) diverts the postcardinal vein from its course and the venous drainage of the mesonephric ridge is assumed by the subcardinal vein. At the same time new longitudinal channels appear and take over intersegmental venous drainage and the whole of the postcardinal vein disappears—except for its extreme cranial and caudal ends. There are at least three such channels on each side, in addition to the postcardinal and subcardinal already mentioned, but, so far as is known, only two of them persist as large vessels in the adult:

- A bilateral longitudinal channel forms dorsolateral to the aorta and **lateral** to the sympathetic trunk and takes over the intersegmental venous drainage from the posterior cardinal vein. This is the *supracardinal* or *thoracolumbar 'complex'*; alternatively *lateral sympathetic line vein*.
- A second channel forms on each side, also dorsolateral to the aorta but **medial** to the sympathetic trunk. This, the *azygos line vein* or *medial sympathetic line vein*, gradually takes over the intersegmental venous drainage from the thoracolumbar line. The intersegmental veins now obviously reach their longitudinal channel by passing medial to the autonomic trunk, the relationship which the lumbar and intercostal veins maintain thenceforth. Cranially the azygos lines join the persistent cranial ends of the posterior cardinal veins.
- Two *subcentral veins* are laid down directly dorsal to the aorta in the interval between the origins of the paired intersegmental arteries. These veins communicate freely with each other and with the azygos line veins, and these connections ultimately form the retro-aortic parts of the left lumbar veins and of the hemiazygos veins.
- Some authorities also recognize a *precostal* or *lumbocostal venous line*, anterior to the vertebrocostal element, and posterior to the supracardinal. A possible derivative is the ascending lumbar vein.

The thoracolumbar or supracardinal veins are, as indicated, lateral to the aorta and sympathetic trunks, which therefore intervene between them and the azygos lines. These veins communicate caudally with the iliac veins and cranially with the subcardinal veins in the neighbourhood of the pre-aortic intersubcardinal anastomosis. In addition, the supracardinal veins communicate freely with each other through the medium of the azygos lines and the subcentral veins. The most cranial of these connections, together with the supracardinal–subcardinal and the intersubcardinal anastomoses, complete a venous ring around the aorta below the origin of the superior mesenteric artery, termed the '*renal collar*' (Huntington 1920 .

The right supracardinal vein persists and forms the greater part of the postrenal segment of the

inferior vena cava, the continuity of the vessel being maintained by the persistence of the anastomosis between the right supracardinal and the right subcardinal in the 'renal collar'. The left supracardinal disappears, but some of the 'renal collar' formed by the left supracardinal–subcardinal anastomosis persists in the left renal vein. It must be added that much confusion and disagreement exists with regard to the disposition, homologies and derivatives of the complicated array of longitudinal veins described above.

In summary, therefore, the inferior vena cava is formed from below upwards by:

- confluence of the common iliac veins
- a short segment of the right postcardinal vein
- postcardinal–supracardinal anastomosis
- part of the right supracardinal vein
- right supracardinal–subcardinal anastomosis
- right subcardinal vein
- a new anastomotic channel of double origin—the hepatic segment of the inferior vena cava
- the cardiac termination of the right vitelline hepatocardiac vein (common hepatic vein).

It should be noted that only the supracardinal part of the inferior vena cava receives the intersegmental venous drainage, and that the postrenal (caudal) segment of the inferior vena cava is on a plane which lies dorsal to the plane of the prerenal (cranial) segment. Thus the right phrenic, suprarenal and renal arteries, which represent persistent mesonephric arteries, pass behind the inferior vena cava, while the testicular or ovarian, which has a similar development origin, passes anterior to it.

In some animals the right postcardinal vein constitutes a large part of the postrenal segment of the inferior vena cava. In these cases the right ureter, on leaving the kidney, passes medially dorsal to the vessel and then, curving round its medial side, crosses its ventral aspect. Rarely, a similar condition is found in the human subject, and indicates the persistence of the right postcardinal vein and failure of the right supracardinal to play its normal part in the development of the vessel.

The ultimate arrangement of some of the embryonic abdominal and thoracic longitudinal cardinal veins may be summarized:

- The terminal part of the left postcardinal vein forms the distal part of the left superior intercostal vein; on the right side its cranial end persists as the terminal part of the vena azygos.


- The caudal part of the subcardinal vein is in part incorporated in the testicular or ovarian vein (McClure & Butler 1925^[4]) and partly disappears. The cranial end of the right subcardinal vein is incorporated into the inferior vena cava and also forms the right suprarenal vein. The left subcardinal vein, cranial to the intersubcardinal anastomosis, is incorporated into the left suprarenal vein. The renal and testicular or ovarian veins on both sides join the supracardinal–subcardinal anastomosis. On the left side this is connected directly to the part of the inferior vena cava which is of subcardinal status through an intersubcardinal anastomosis.
- The right supracardinal vein forms much of the postrenal (caudal) segment of the inferior vena cava. The left supracardinal vein disappears entirely.
- The right azygos line persists in its thoracic part to form all but the terminal part of the vena azygos. Its lumbar part can usually be identified as a small vessel which leaves the vena azygos on the body of the twelfth thoracic vertebra and descends on the vertebral column, deep to the right crus of the diaphragm, to join the posterior aspect of the inferior vena cava at the upper end of its postrenal segment. The left azygos line forms the hemiazygos veins.
- The subcentral veins give rise to the retro-aortic parts of the left lumbar veins and of the hemiazygos veins.

Veins of the Head

The veins of the head have a complicated developmental history (3.190^[5]), and consult Markowski 1911^[6]; Streeter 1918^[7]; Padget 1957^[8]; Butler 1957^[9], 1967^[10]; Browder & Kaplan 1976^[11]). The primary vessels consist of a close-meshed capillary plexus drained on each side by the precardinal vein, which is at first continuous cranially with a transitory *primordial hindbrain channel*, lying on the neural tube medial to the cranial nerve roots. This is soon replaced by the *primary head vein* which runs caudally from the medial side of the trigeminal ganglion, lateral to the facial and vestibulocochlear nerves and otocyst, then medial to the vagus nerve, to become continuous with the precardinal vein. A lateral anastomosis subsequently brings it lateral to the vagus nerve. The cranial part of the precardinal vein forms the internal jugular vein; its caudal moiety has already been described on page 324^[12].

The primary capillary plexus of the head becomes separated into three fairly distinct strata by the differentiation of the skull and meninges. The superficial vessels, draining the integument and underlying soft parts, eventually discharge in large part into the *external jugular system*. They retain some connections with the deeper veins through so-called emissary veins. Deep to this is the *venous plexus of the dura mater*, from which the dural venous sinuses differentiate. This plexus converges on each side into *anterior, middle* and *posterior dural stems*. The anterior stem drains the prosencephalon and mesencephalon entering the primary head vein rostral to the trigeminal ganglion. The middle stem drains the metencephalon and empties into the primary head vein caudal to the trigeminal ganglion, while the posterior stem drains the myelencephalon into the commencement of the precardinal vein (3.98A,B^[13]). The deepest capillary stratum is the pial plexus from which the veins of the brain differentiate; it drains at the dorsolateral aspect

of the neural tube into the adjacent dural venous plexus. In addition the primary head vein also receives, at its cranial end, the *primitive maxillary vein*, draining the maxillary prominence and region of the optic vesicle.

The vessels of the dural plexus undergo profound changes, largely accommodating the growth of the cartilaginous otic capsule of the membranous labyrinth and expansion of the cerebral hemispheres. With growth of the otic capsule the primary head vein is gradually reduced and a new channel joining anterior, middle and posterior dural stems appears dorsal to the cranial nerve ganglia and the capsule (Butler 1967^[4]). Where this new vessel joins the middle and posterior stems, together with the posterior dural stem itself, is formed the adult *sigmoid sinus* (3.190B ) .

Between the growing cerebral hemispheres and along the dorsal margins of the anterior and middle plexuses there forms a curtain of capillary veins, the *sagittal plexus*, in the position of the future falx cerebri. Rostrodorsally this plexus forms the *superior sagittal sinus* and is continuous behind with the anastomosis between the anterior and middle dural stems, which forms most of the *transverse sinus*. Ventrally the sagittal plexus differentiates into the *inferior sagittal* and *straight sinuses* and the *great cerebral vein*, and drains, more commonly, into the left transverse sinus. (For right/left variations in the terminations of sagittal and straight sinuses, and occurrence of a confluence, see p. 1583^[4].)

The vessels along the ventrolateral edge of the developing cerebral hemisphere form the transitory *tentorial sinus*, which drains the convex surface of the cerebral hemisphere and basal ganglia, and the ventral aspect of the diencephalon, to the transverse sinus. With expansions of the cerebral hemispheres, and in particular the emergence of the temporal lobe, the tentorial sinus becomes elongated, attenuated and eventually disappears, and its territory is drained by enlarging anastomoses of pial vessels which become the *basal veins*, radicles of the great cerebral vein.

The anterior dural stem disappears and the caudal part of the primary head vein dwindles; it is represented in the adult by the *inferior petrosal sinus*. The cranial part of the primary head vein, medial to the trigeminal ganglion, persists and still receives the stem of the primitive maxillary vein. The latter has now lost most of its tributaries to the anterior facial vein, its stem becoming the main trunk of the *primitive supra-orbital vein*, which will form the *superior ophthalmic vein* of the adult. Thus, the main venous drainage of the orbit and its contents is now carried via the augmented middle dural stem, the *pro-otic sinus*, into the transverse sinus and at a later stage into the cavernous sinus. The *cavernous sinus* is formed from a secondary plexus, derived from the primary head vein and lying between the otic and basi-occipital cartilages. This forms the inferior petrosal sinus and drains through the primordial hindbrain channel into the internal jugular vein. The *superior petrosal sinus* arises later from a ventral metencephalic tributary of the pro-otic sinus; it communicates secondarily with the cavernous sinus (Butler 1957^[4], 1967^[4]). The pro-otic sinus meanwhile has developed a new and more caudally situated stem draining into the sigmoid sinus; this new stem is the *petrosquamosal sinus* (p. 1584^[4]) and the pro-otic sinus becomes, with progressive ossification of the skull, diploic in position. The development of the venous drainage and portal system of the hypophysis cerebri is closely associated with that of

the venous sinuses (Wislocki 1937¹; Niemineva 1950², see also p. 1883³).

Cerebral Veins

These can be identified from 16 weeks onwards; the *superior, middle, inferior, anterior* and *posterior cerebral veins* are developed and appear more tortuous than the meningeal arteries.

Veins draining the cortex, white matter and deeper structures are recognized in the midtrimester. *Subcortical veins* drain the deep white matter, deep cortical and subcortical superficial tissue; they terminate together, with *cortical veins* which drain the cortex, in the *meningeal veins*. The deep white matter and central nuclei are drained by longer veins, which meet and join subependymal veins from the ventricular zone. Anastomoses between various groups of cortical veins can be recognized by 16 weeks gestation. The *inferior anastomotic vein (of Labbe)*, an anastomosis between the middle cerebral and inferior cerebral veins, becomes recognizable at 20 weeks but the *superior anastomotic vein (of Trolard)*, connecting the superior and middle cerebral veins, appears not earlier than the end of 30 weeks.

Rapid cortical development is correlated with the regression of the middle cerebral vein and its tributaries and development of ascending and descending cortical veins and intraparenchymal (medullary) arteries and veins.

Cerebral venous drainage in a full-term baby is essentially composed of two principal venous arrays, the *superficial veins* and the *deep Galenic venous system* with anastomoses between these two systems persisting into adult life (Wigglesworth & Pape 1978⁴).

Venous Drainage of the Face, Scalp and Neck

This becomes established after the development of the skull. The first identifiable vessel is the *ventral pharyngeal vein*, draining the massive mandibular and hyoid arches into the common cardinal vein. With the elongation of the neck its termination is transferred to the cranial part of the precardinal vein which later becomes the *internal jugular*. The ventral pharyngeal vein, receiving tributaries from the face and tongue, becomes the *linguofacial vein*. With development of the face the primitive maxillary vein extends its drainage into the territories of supply of the ophthalmic and mandibular division of the fifth nerve, including the pterygoid and temporal muscles, and over the lower jaw it anastomoses with the linguofacial vein. This anastomosis becomes the *facial vein*; it receives a strong *retromandibular vein* from the temporal region, and drains through the linguofacial vein into the internal jugular. The stem of the linguofacial vein is now the lower part of the facial vein, whilst the dwindling connection of the facial with the primitive maxillary becomes the *deep facial vein*. The *external jugular vein* is developed from a tributary of the cephalic vein from the tissues of the neck and anastomoses secondarily with the anterior facial vein. At this stage the *cephalic vein* forms a venous ring around the clavicle from which it is connected with the caudal part of the precardinal. The deep segment of the venous ring forms the *subclavian vein* and receives the definitive external jugular vein. The superficial

segment of the venous ring dwindles, but may persist in adult life (Padget 1957^[4]).

Veins of the Limbs

At the tip of the early limb bud, blood in the terminal capillary plexus returns to the body via a *marginal vein* that develops along the pre- and postaxial borders of the limb. The marginal vein is separated from the overlying ectoderm by an avascular zone of mesenchyme (p. 319^[4]). As the limb enlarges the marginal vein can be subdivided into *pre-* and *postaxial veins* running along their respective borders. These latter vessels are the precursors of the superficial veins of the limb. Generally the preaxial (superficial) veins join to deep veins at the *proximal joint*, and the postaxial (superficial) veins join to deep veins at the *distal joint* of the limb. Deep veins develop in situ alongside the arteries.

In the upper limb the preaxial vein becomes the *cephalic vein*; it drains at the shoulder into the axillary vein. The postaxial vein becomes the *basilic vein*, which passes deep in the arm to continue as the axillary vein.

In the lower limb the preaxial vein becomes the *great saphenous vein* and drains at the saphenous opening into the femoral vein. The postaxial vein becomes the *short saphenous vein*; this passes deep to join the popliteal vein.

Lymphatic and Lymphoid System

Two different views are current as to the initial stages in the development of the lymphatic system (Rusznayák et al 1960^[4]). According to the first view (Huntington 1908^[4]; McClure & Butler 1925^[4]) lymphatic spaces commence as clefts in the mesenchyme, and their lining cells take on the characteristics of endothelium (Kampmeier 1969^[4]). These spaces form capillary plexuses from which certain *lymph sacs*, to be noted later, are derived. The connections of the lymphatic and venous systems are regarded as entirely secondary. In contrast, however, according to Sabin (1912^[4]), the earliest lymph vessels arise as capillary offshoots from the endothelium of the veins, as capillary plexuses. These plexuses lose their connections with the venous system and become confluent to form lymph sacs. The balance of the evidence suggests that all but the earliest lymphatic channels originate independently of the venous system and only acquire connections with it at a later stage (Kampmeier 1969^[4]).

In the human embryo the lymph sacs from which the lymph vessels are derived are six in number: two paired (the *jugular* and the *posterior lymph sacs*) and two unpaired (the *retroperitoneal* and the *cisterna chyli*). In lower mammals an additional pair (the *subclavian*) is present, but in the human embryo these are merely extensions of the jugular sacs.

The position of the sacs is as follows (3.191^[4]):




- the *jugular*, the first to appear, at the junction of the subclavian vein with the


precardinal, with later prolongations along the internal and external jugular veins



- the *posterior* encircling the left common iliac vein
- the *retroperitoneal*, in the root of the mesentery near the suprarenal glands
- the *cisterna chyli*, opposite the third and fourth lumbar vertebrae.

From the lymph sacs the lymph vessels bud out along lines corresponding more or less closely with the course of embryonic blood vessels, most commonly veins, but many arise *de novo* in the mesenchyme and establish connections with existing vessels. In the body wall and that of the intestine the deeper plexuses are the first to be developed; by continued growth of these, the vessels in the superficial layers are gradually formed.




The *thoracic duct* is, phylogenetically, a bilateral structure. In man it comprises the caudal part of the right vessel, a transverse anastomosis and the cranial part of the left vessel. According to the second view cited above, it is formed from anastomosing outgrowths from the jugular sacs and cisterna chyli. At its connection with the cisterna it is at first double, but the vessels soon join. Numerous valves are laid down in the duct during the fifth month, but many of them disappear prior to birth. Those which persist are formed in situations where the duct may be subjected to pressure, for example where it is crossed by the oesophagus and the aortic arch.

All the lymph sacs except the cisterna chyli (see p. 1609) are, at a later stage, divided by a number of slender connective tissue bridges. Subsequently they are invaded by lymphocytes and transformed into groups of lymph nodes, the lymph sinuses representing portions of the original cavity of the sac. The caudal part of the cisterna chyli is similarly converted, but its rostral part sometimes persists as a definitive cisterna; in many cases the cisterna chyli is plexiform (p. 1610). The siting of the major groups of lymph nodes follows a similar basic pattern amongst the mammals (Spira 1962)

Despite the wide array of labelling techniques for histology, there is a paucity of recent anatomical studies on the development of the lymphatic system. Experimental studies have shown that loading of the vascular system with fluid increases the thoracic lymph duct flow in fetal sheep, suggesting that the rate of flow is an important safety factor against fetal oedema (Brace 1993)

Haemal lymph nodes are said to develop as mesenchymal condensations in close relation to blood vessels rather than lymphatics (Meyer 1917; Turner 1969)

Spleen

The spleen (3.76, 78D, 79) appears about the sixth week as a localized thickening of the coelomic epithelium of the dorsal mesogastrium near its cranial end, and the proliferating cells invade the underlying angiogenetic mesenchyme, which becomes condensed and vascularized. The process occurs simultaneously in several adjoining areas which soon fuse to

form a lobulated spleen of dual origin from coelomic epithelium and from mesenchyme of the dorsal mesogastrium. With enlargement, the spleen projects to the left so that its surfaces are covered by the peritoneum of the mesogastrium on its left aspect, thus forming a boundary of the general extrabursal (greater) sac. When fusion occurs between the dorsal wall of the bursa omentalis and the dorsal parietal peritoneum, fusion does not extend to the left as far as the spleen (3.78D, 79), which remains connected to the dorsal abdominal wall (left kidney and suprarenal) by a short lienorenal ligament, while its original connection with the stomach persists as the gastrosplenic ligament. The lienorenal ligament contains the tail of the pancreas. The earlier lobulated character of the spleen disappears, but is indicated by the presence of notches on its upper border in the adult.

The histogenesis of the spleen has attracted relatively little attention. For earlier accounts consult references (Sabin 1912; Thiel & Downey 1921; Lewis 1956; von Herrath 1958; Bloom & Fawcett 1975). The vascular reticulum is well developed at 8 to 9 weeks, with immature reticulocytes and numerous closely spaced thin-walled vascular loops. Differentiation of blood cells, macrophages, and of arteries, veins, capillaries and sinusoids has occurred by the eleventh to twelfth week. The capsule consists at first of cuboidal cells bearing cilia and microvilli (Weiss 1957).

The spleen is subject to various anomalies, including complete *agenesis*, multiple spleens or *polysplenia*, isolated small additional *spleniculi* and persistent lobulation. Attention has been directed to association of cardiac, pulmonary and other abnormalities with asplenia or polysplenia (Rose et al 1975).

For development of the thymus see page 176.

Prenatal Growth in Form and Size

The absolute size of neither embryo nor fetus affords a reliable indication of either its true age or stage of structural organization, even though graphs based on large numbers of observations have been constructed to provide averages. All such data suffer from the difficulty of equating dimensions and degree of differentiation with the actual time of conception, which can rarely, if ever, be established with complete exactness. The life of the individual really commences with fertilization, but the date of this cannot be exactly determined in mankind. It has long been customary to compute the age, whether in a normal birth or an abortion, from the first day of the last menstrual period of the mother but, since ovulation usually occurs near the fourteenth day of a period, this 'menstrual age' is about two weeks too much (see p. 92). Where a single coitus can be held to be responsible for conception, a 'coital age' can be established and the 'fertilization age' cannot be much less than this, because of the limited viability of both gametes; but it is usually held that the difference may be several days—a highly significant interval in the earlier stages of embryonic development. Even if the time of ovulation and coitus were known in instances of spontaneous abortion, not only would some uncertainty still persist with regard to the time of fertilization but there would also remain an indefinable period between the cessation

of development and the actual recovery of the conceptus. With the legalization of abortion in some countries the latter source of inaccuracy may be expected to become less important.

To overcome these difficulties early embryos have been graded or classified, on the basis of both internal and external features, into developmental stages or 'horizons' (3.192). Classic contributions in this field have been made by Lillie (1917), Streeter (1942, 1945, 1948, 1949), Hamilton (1944), Hertig et al (1956), Heuser & Corner (1957), and O'Rahilly and others between the years 1963 and 1987. Although it has become customary to describe these developmental levels as stages, the earlier descriptions are still accepted. However, Stages 1 to 9, covering the first three weeks of development, have been given a more reliable basis by O'Rahilly (1973), who has also gathered together the pertinent literature. Details on stages 1–23 are provided by O'Rahilly and Muller (1987), who describe developmental time in terms of postovulatory days. In each case the range is ± 1 day for stages 1–14; thereafter, the ages are greater than initially described by Streeter. Readers should consult the charts on pp. 45 and 50.

Stages 1 to 3

These occupy the first 4–5 days after fertilization of the oöcyte in the ampulla of the uterine tube. The initial 24 hours (Stage 1) are occupied by fertilization, the dominant feature of which is the fusion of the male and female pronuclei. This is followed by the first mitotic division, which is the onset of segmentation, or cleavage, and is arbitrarily regarded as the transition from Stage 1 to Stage 2. Stage 2 is characterized by the continuation of cleavage, starting with two blastomeres and ending with about 12. During this stage the developing morula moves along the uterine tube, by mechanisms still not wholly understood, a journey occupying about four days. During the fourth day a segmentation cavity appears within the morula and this is taken as initiating Stage 3, which thus corresponds to the establishment of a free blastocyst.

Although comparatively few human embryos representing Stages 1 to 3 have been recovered (see p. 136 and consult O'Rahilly 1973), a considerable degree of agreement exists and, moreover, current observations of in vitro fertilization between human spermatozoa and ova (3.192A–H) support the earlier descriptions.

Stages 4 to 6

These are concerned with the endometrial attachment of the blastocyst, trophoblastic development, implantation, further development of the blastocyst and the appearance of the primitive streak (3.30, 35, 36, 38, 39). Stage 4 corresponds to the *fifth and sixth postfertilization days*. The blastocyst, now in the uterine cavity, loses its zona pellucida and it begins the rapid but complex activities of orientation in respect of the endometrium, adhesion, penetration and the cellular proliferation of trophoblastic growth. This establishment of dependence on the maternal circulation for nutritional requirements is far more rapid in primates than in other mammals. Stage 5 is reached when implantation has occurred, occupying the *seventh to twelfth days*; syncytiotrophoblastic and cytotrophoblastic strata have differentiated,

the proamniotic cavity has appeared and a labyrinthine system of intercommunicating trophoblastic lacunae, through which the maternal blood ebbs and flows, has developed. A little later in Stage 5 the exocoelomic membrane has been identified. In Stage 6 chorionic villous stems become defined and begin to develop side branches almost at once, producing an increasingly complex intervillous space. A little later the primitive streak becomes apparent and differentiation of the embryonic area has commenced and may now be distinguished from the various extraembryonic tissues.

Because of the complexity of the events in Stages 5 and 6, both have been subdivided by some authorities. A much greater number of human embryos have been recovered which represent Stages 4 to 6.

Stages 7 to 9

Characterized by basic embryogenic changes, during *Stage 7 (approximately sixteenth postovulatory day)* the primitive streak develops further and the notochordal ('head') process appears, together with the other mesenchymal strata. The chorion and amnion continue to develop, villous stems being generally distributed over the former but more pronounced at the embryonic pole. Haemopoietic foci appear in the wall of the definitive yolk sac, and the cloacal membrane and allanto-enteric diverticulum are defined. Associated with the latter primordial germ cells have been noted. In *Stage 8 (seventeenth to nineteenth day)* the prechordal plate, primitive pit, neural groove and notochordal and neurenteric canals are all definable. By *Stage 9 (nineteenth to twenty-first day)* the neural groove is deepening and the first somites begin to appear about midway along it. The cranial half of the groove, representing developing brain, begins to develop a cephalic flexure, optic primordia become visible and early head and tail folds have appeared, as Stage 10 is approached. The foregut is becoming defined, and early pharyngeal pouches may be identified. The embryo is now 1.5–2.0 mm in length.

Stages 10 to 12

Occupying *days 21–23, 23–25, 25–27* respectively, these stages feature continued formation of somites and, during this, the fourth week, head and tail folds are completed, the neural groove closes and primary cerebral vesicles appear. The cervical flexure can now be recognized, the optic vesicles form and the lental and otic placodes appear and become vesicular. The pharyngeal arches are appearing, lateral folds are more clearly defined and the cloacal membrane and hindgut are becoming distinct. Rudimentary limb buds appear and the heart tubes fuse into a common loop in which contractile activity commences. The primordia of the thyroid gland, lungs, liver, pancreas and mesonephric tubules are all identifiable. The embryo is about 4.0 mm in length.

Stages 13 to 15



Corresponding approximately to the *fifth week* of development (28, 32 and 33 *days*), at these stages the embryo grows from about 4 mm to 8 mm. It becomes markedly curved and its junction

with the yolk sac relatively constricted. The cervical flexure is increased and the mesencephalic flexure is appearing. The dorsolateral (alar) and ventrolateral (basal) laminae are differentiating, and the emerging corpus striatum, thalamus epithalamus and hypothalamus are loci of proliferating cells. The cranial and spinal nerves are developing, together with their ganglia, from the associated placodes and neural crest elements. The limb buds are elongating, displaying joint flexures; the rudimentary hands and feet are differentiating. The olfactory placodes, maxillary, mandibular and frontonasal prominences, tongue primordia and the hypophyseal pouch (of Rathke) are all appearing. The tubotympanic recesses are defined and the primordia of the thymus and parathyroid glands can be identified. In the developing heart the septum primum appears and its cornua define the foramen primum. The mesonephric ducts reach the cloaca and subsequently the metanephric (ureteric) buds appear and extend to the metanephrogenic masses of mesoderm in the sacral nephrogenic cord. The gonadal ridges, urorectal septum and genital tubercle are also developing.

Stages 16 to 20

Roughly equivalent to the sixth and seventh weeks, (37, 41, 44, 47–48, 50–51 days), by the end of these stages the embryo has a length of about 13–15 mm. Its curvature has further increased and its head and relatively long tail are in contact with the developing umbilical stump. The pontine flexure, cerebral hemispheres and cerebellum are developing. The upper limbs and the facial region are growing and differentiating rapidly; the palatal processes and primitive nasal prominences are apparent and the oronasal membrane ruptures. The liver produces a surface prominence between the cardiac region and the umbilical cord. Into the latter the midgut loop herniates and the appendix and caecum become distinguishable in it. The spleen develops, as do the paramesonephric (Mullerian) ducts. The foramen primum in the heart closes, with simultaneous opening of the foramen secundum and septation of the bulbus cordis. Cardiac muscle is differentiating. Haemopoiesis commences in the liver. Chondrification of many skeletal elements begins and ossification commences in mesenchymatous bones, the mandible and clavicles.

Stages 21 to 23

Completing the series (52, 54, 56–67 days), these stages represent the major part of the eighth week when the *formative* or *embryonic period* is regarded as coming to an end (3.192 ). During these stages there is a remarkable change in the external appearance of the embryo, for at the beginning of this period the individual still appears markedly 'embryonic', though clearly a primate, whereas at the end the form is most definitely human. The head is less flexed and the neck longer and clearly defined. The development of the face proceeds much further, with completion of the upper lip and nostrils, although the latter are plugged and the palate still incomplete. Enamel organs are developed from the dental laminae. The external ears and the eyelids are developing and the limbs elongate considerably, approaching much nearer their ultimate proportions and displaying well-formed hands and feet with separated digits. Early in this period the interventricular septum is completed. Skeletal and visceral muscle tissues begin to differentiate about this time, and generalized ossification occurs in enchondral bones. The onset of marrow formation in the humerus occurs at stage 23. This was adopted by Streeter (1949 ) as

the conclusion of the embryonic and the beginning of the fetal period of prenatal life. Other systemic developments reached by this stage include vesicles in the metanephrogenic mass; the remainder of the nephrons and the collecting tubules of the kidneys are defined. The ovaries or testes are distinguishable and the paramesonephric (Mullerian) ducts are fusing to form the primordia of uterus and vagina. The external genitalia are further advanced and show sexual differentiation by the beginning of the eighth week. The cloacal membrane becomes perforate and the tail is retrogressing. By the end of the period the embryo possesses almost all the structural features, internal and external, characteristic of the human mammal and it now passes into the fetal period. The embryonic period, during which *patterned differentiation* occurs with consequent *organogenesis*, tends to overshadow the growth which accompanies these events. Very considerable increase in size has, however, occurred; from a single cell about 0.14 mm in diameter the embryo has become a most complex and functioning creature, consisting of millions of cells and with a length of about 30 mm or more, and it has increased in weight many thousands of times. During the *fetal period*, which occupies the third to tenth lunar months (3.193👁️, 194👁️), the accent is upon growth rather than differentiation but, of course, the latter continues throughout this period and to a lesser degree after birth (and in some tissues, throughout life). During this period the overall rate of growth in length is greater but not markedly so; from the fourth to sixth weeks the rate is about 1 mm per day, with a maximum of about 2 mm during the fourth month. The increase in length in the fetal period is from 30/40 to about 500 mm, and the increase in weight from perhaps 2 or 3 g to more than 3000 g.

Third Month

During this month head flexion decreases further and the neck becomes proportionately longer. The eyelids meet and fuse and will remain temporarily united until the sixth lunar month. Nails appear on the digits and the upper limbs in general are comparatively accelerated in development. The umbilical protrusion of the gut is reduced—accompanying a proportionate augmentation of abdominal volume.

Fourth and Fifth Months

The covering of primary hair—the *lanugo* (see p. 405📖) appears during this month; the head and upper limbs are still disproportionately large and, although the trunk and lower limbs begin to catch up by increased rates of growth during the rest of uterine life, the same disproportion is present after birth and to a diminishing degree throughout childhood and on into the years of puberty. By the end of the fourth month the eyes have moved even further into an anteriorly directed position, but are still relatively wide apart. The external ear is approaching its characteristic form and is nearer its ultimate position, at the side of the head and no longer in the upper part of the neck. The total fetal length, including the lower limb, is now of the order of 230 mm. Its weight at the end of the *fifth month* is about 300 g, which will be increased more than tenfold during the second half of intrauterine life. Towards the end of this period sebaceous glands become active, and the sebum secreted blends with desquamated epidermal cells to form a cheesy covering to the skin, the *vernix caseosa*, usually considered to protect the former from maceration by the amniotic fluid. During this month the mother becomes conscious of fetal

movement—so-called 'quickening'.

Sixth Month

This month witnesses a further general change of bodily proportions and facial appearance towards those of the infant at birth. The lanugo darkens and the skin becomes markedly wrinkled, presumably through a disparity in the growth rates of cutaneous and subcutaneous tissues. The eyelids and eyebrows are now well developed. The vernix caseosa is more abundant. The length of the fetus is about 300 mm by the end of this month.

Seventh Month


During this month the hair of the scalp is lengthening and the eyebrow hairs and the eyelashes are well-developed. The eyelids themselves separate and the pupillary membrane disappears. The body becomes more plump and rounded in contour and the skin loses its wrinkled appearance due to increased deposition of subcutaneous fat. Towards the end of this month the fetus is viable, without the technological assistance found in 'special baby units', and may be successfully raised if born prematurely. Its length has increased to about 350 mm and it weighs about 1.5 kg.

Remaining Lunar Months

Throughout the remaining lunar months of normal gestation the covering of vernix caseosa is prominent. There is a progressive loss of lanugo, except for the hairs on the eyelids, eyebrows and scalp. The bodily shape is becoming more infantile, but despite some acceleration in its growth the leg has not quite equalled the arm in length proportionately even at the time of birth. The thorax broadens relative to the head, and the infra-umbilical abdominal wall shows a relative areal increase, so that the umbilicus gradually becomes more centrally situated. Average lengths and weights for the eighth, ninth and tenth months are 40, 45 and 50 cm and 2, 2.5 and 3–3.5 kg.

Tenth Lunar Month

At the end of this month, just before birth, the lanugo has almost disappeared, the umbilicus is central and the testes, which begin to descend with the vaginal process of peritoneum during the seventh month and are approaching the scrotum in the ninth month, are usually scrotal in position. The ovaries are not yet in their final position at birth; although they have attained their final relationship to the uterine folds, they are still above the level of the pelvic brim.

The length of the period of gestation is regarded as nine calendar months in obstetric practice—approximately 270 days. It is usually about 266 days—10 lunar months less 14 days (see p. 344 ).

Congenital Malformations and Prenatal Diagnosis

Introduction

Infants have always been born with defects or anomalies. In early societies those surviving the neonatal period with developmental defects were often considered 'monsters'; they were viewed as throwbacks to undeveloped or underdeveloped types of humans, or thought to be a punishment to the mother or family. Records of human congenital malformations, in cave-paintings, sculptures and ultimately in writings, extend backwards into prehistory showing talipes, achondroplasia and conjoined twins, portrayed together with centaurs, sirens, mermaids and other fanciful creatures (Barrow 1971^[1]). The Hippocratic School identified hydrocephalus.

It was not until the seventeenth and eighteenth centuries that Harvey, Wolff, von Haller, the Hunters and their contemporaries, stimulated by the growing knowledge of embryology, initiated the *theory of embryonic arrest* to explain malformations. Saint-Hilaire experimented on developing chick embryos in the early nineteenth century, and with this approach the study of teratology (the origins and production of monsters) was consolidated as a science.

A commonly held belief that the experiences of the pregnant mother, usually visual, could influence her unborn offspring in an adverse manner is scarcely dead even today. However, that factors in the **maternal environment** may influence the embryo has proved true, but in a different sense, for Watson, as long ago as 1749, suggested that fetal disease, contracted by a transplacental route, might be a cause of congenital abnormality, citing variola as an example. This theory slowly dwindled, and was almost extinguished a century later by the authority of Virchow and His, whose views dominated teratological opinion through most of the second half of the nineteenth century. A contemporary, working in obscurity, was Mendel; as his work became known and genetics flowered into the twentieth century attention inevitably turned to the hereditary aspect of congenital defects (already foreshadowed by Paré and John Hunter, centuries earlier). In 1941 Gregg's observation that congenital cataract is associated with the infection of pregnant mothers by rubella revived interest in environmental factors.

Teratogenic Causes

The expansion of experimental embryology has revealed a wide array of environmental agents capable of affecting normal development, including temperature variations, mechanical insult, variation in substances such as lithium and magnesium in culture media, exposure to irradiation, hypoxia, hypo- and hypervitaminosis, hormonal effects (especially with oestrogens and androgens), nutritional defects and exposure to various drugs and other chemicals. The identification of human teratogens may be said to have commenced with Gregg's demonstration of the effects of rubella virus. Other viral and bacterial maternal infections have since been implicated, especially cytomegalovirus and toxoplasmosis (see **Table 3.2**^[2]). But with the multiplication of drugs, for such purposes as abortion (aminopterin) and sedation (thalidomide),

some of which have proved tragically teratogenic, teratological research has been much concentrated upon drugs.

A very large number of cytotoxic agents are now known, and many have been used as experimental teratogens, mostly on rodents. Some act as antimetabolites, amino-acid antagonists, antipurines or spindle toxins and most are highly selective in their effects, which are produced only by controlled dosage at specific periods during development. Wilson and Warkany (1965) formalized a methodology for screening drugs for teratogenicity in animals. Connors (1975^[4]) emphasized the complex array of variable parameters which help to determine whether an agent acts as a teratogen or not. These include embryonic age, the amount, route and mode of administration of the agent, placental and embryonic permeability, maternal or embryonic ability to inactivate the agent, the state of differentiation of target cells and their ability to recover. Sullivan (1975^[4]) reviewed the literature concerning teratogenic drugs taken by pregnant women, classifying them on their sites of action, whether directly on the embryo (thalidomide, tetracycline antibiotics), on embryonic endocrine balance (oestrogens and androgens), on the placenta or on maternal tissues. These considerations are, of course, of intense clinical importance, but their contribution to explanations of teratogenic mechanisms is limited, except in so far as some drugs are known to be teratogenic at specific stages of development.

A chart of the known infectious and chemical teratogens is shown in [Table 3.2](#) ^[4].

Genetic Causes

It has long been known that chromosomes and genes which regulate developmental processes may themselves be defective. The ways in which such defective chromosomes or genes may interact with other normal chromosomes, with the normal environmental conditions during development, or with abnormal environmental conditions, for example in infections or exposure to drugs, is not yet known. Study of such mechanisms forms the basis of genetic approaches to developmental perturbations and congenital malformations.

Genetic conditioning of congenital abnormalities whether structural, metabolic or behavioural has been the subject of very widespread research or observation. That alterations in the **number of chromosomes** can cause malformations was demonstrated in the 1950s, firstly when Tjio and Levan (1956^[4]) established the number of chromosomes in humans to be 46, and later in 1959^[4] when Lejeune et al showed a tripling (trisomy) of chromosome 21.

From 1970 techniques became available for studying chromosomal banding and thus smaller aberrations of chromosomal structure. By 1989 in excess of 600 abnormalities of chromosomal structure had been described.

The first human anomaly to be identified as genetic was alkaptonuria (Garrod 1902^[4]), a rare condition in which patients have arthritis and urine which darkens on standing. It is now known to be a *single gene defect* causing deficiency of a single enzyme (homogentisic acid). Five enzyme defects had been identified by 1959 and that number had reached 200 by 1992. Genes

can now be assigned to individual chromosomes. First those genes linked to the X chromosome were discovered. Later genes were mapped to the autosomes commencing with the thymidine kinase gene to chromosome 17 in 1967. So far 2316 genes have been mapped to their respective chromosomes and 40% of the Mendelian traits have been identified (Connor & Ferguson-Smith 1993¹).

The majority of congenital malformations seen in term neonates have not yet been linked either to specific chromosome aberrations or to single gene defects. This large group of malformations is believed to be caused by a number of pairs of genes producing additive effects which accrue until a threshold is passed; then morphological anomalies, which cannot be corrected by catch-up growth, result. Such malformations are termed *multifactorial disorders*. The number of genes involved is not known nor the mechanisms by which they interact with each other or the environment. More than 20 discontinuous multifactorial traits have been described; some cause congenital defects while others produce conditions seen commonly in adult life.

With the genetic and physical mapping reagents now becoming available considerable advances have been and are being made in the identification and localization of disease-genes. An impetus for this line of research was provided by The Human Genome Project (formally started in 1990), an international research effort to analyse the structure of human DNA and to determine the location of the estimated complement of about 100 000 human genes. So far about 2.3% of this total has been mapped. The Human Genome Project also includes the analysis of DNA from a set of non-human model species to provide comparative information about conserved gene action and how the human genome functions. For a review of the advances of the Human Genome Project see Guyer and Collins (1995²).

Vulnerable Periods of Development

Information about the stage of gestation at which anomalies first appear has been obtained both by the correlation of malformations of particular organs and systems with the time at which those organs and systems develop, and by the direct experimental perturbation of development in animals to produce malformations similar to those seen in human infants. The time at which major organs develop can be indicated on a developmental scale (3.195³). This allows examination of the state of development of each system at a given time. Although organogenesis is traditionally described for each system, all systems develop at the same time, within a very short period of the total gestation time. Thus, in 3.195³, it can be seen that little embryonic development occurs in the first two weeks (postovulatory) of gestation, when extraembryonic structures develop. Organogenesis occurs mainly between weeks 2 and 8, after which time tissue differentiation, growth and maturation continue to 38 weeks (see 3.195³).

Teratogenic insults may be embryotoxic or cause disruption of those systems undergoing major proliferative and morphological change. The time of the insult will be reflected in the systems affected. Genetic defects will affect earlier stages than teratogenic insults and may similarly result in abortion, but may also produce specific defects of tissue differentiation and genotypes with multiple anomalies which survive until term. Late malformations may be caused by

mechanical effects.

Nomenclature

The identification of a developmental defect requires a description of the condition and an underlying knowledge of the development of the system or systems involved. In an attempt to standardize the descriptive terminology for birth defects, Spranger et al (1982¹) subdivided the all embracing title 'birth defect' into specific groups and recommended appropriate descriptive terms and their usage. The following terms were suggested:

- A *field defect* describes a collection of malformations affecting a developmental field, i.e. parts of an embryo which respond as a co-ordinated unit to a disturbance in development, or organs and tissues developing from a common origin, for example the neural crest. The latter may cause widespread (polytopic) effects.
- A *malformation* is defined as a primary structural defect of an organ or part of an organ resulting from an inherent abnormality in development, for example isolated, non-syndromal facial clefting or congenital heart disease.
- A *deformation* is a defect caused by an external mechanism affecting a normally formed organ or structure, for example talipes secondary to oligohydramnios.
- A *disruption* describes abnormal development as a result of external interference, which may be caused by: a *teratogen*—phenytoin causing distal limb hypoplasia; an *infection*—rubella causing cataracts; *trauma*—amniotic bands causing amputation.
- A *dysplasia* is defined as an abnormal organization of cells into a tissue, for example osteogenesis imperfecta. Many dysplasias are genetic and carry high recurrence risks for siblings and/or offspring, for example in skeletal defects like achondroplasia.
- A *sequence* describes multiple anomalies resulting from a single factor; for example, Potter sequence—here chronic leakage of amniotic fluid (or low urinary output)—leads to oligohydramnios which in turn leads to fetal compression causing talipes, dislocation of the hips, squashed facies and pulmonary hypoplasia.
- A *syndrome* is defined as a pattern of anomalies known to be causally related which do not constitute a sequence or a polytopic field defect. A syndrome can be caused by a chromosome abnormality, for example Down's syndrome; a single gene defect, for example Meckel syndrome; or an environmental agent, for example fetal alcohol syndrome.
- An *association* describes the non-random occurrence of multiple anomalies which fit none of the above definitions. Such associations are often described as an acronym; for example the VATER association includes Vertebral, Anal, Tracheo-Esophageal and Renal anomalies.

Congenital anomalies are also described as 'major' or 'minor'. A major defect is one which results in mortality or significant morbidity, whereas a minor defect would not affect normal life

expectancy; an example of the latter would be a supernumerary nipple. However, it has been noted that the occurrence of an isolated minor malformation may indicate a more serious underlying problem, and that multiple minor malformations may be the only external features of an underlying syndrome (Young 1992^[4]).


Incidence

It may be stated with some veracity that defects of the genome account for the vast majority of spontaneous abortions in early pregnancy, defects seen at birth, defects in childhood and a range of adult disorders. The production of an appropriate genome is the first essential for an individual and later the satisfactory interaction of a particular genome with its environment can be described as 'health'. Thus examination of the statistics for congenital anomalies shows, in the main, the incidence of inappropriate genome production and the consequences of aberrant genome expression surviving to birth and childhood.

At least 15% of all recognized pregnancies result in spontaneous abortion before 12 weeks gestation, with 80% of spontaneously aborted embryos having major disturbances of development, often as a result of a non-viable cytogenetic abnormality (Young 1992^[4]). Chromosomal abnormalities occur in approximately 40% of unselected spontaneous abortions (Hsu 1986^[4]); of these autosomal trisomy accounts for 52%, monosomy X 19%, triploidy 16%, and tetraploidy 6%. Of the fetuses spontaneously aborted in the second trimester of pregnancy, up to 20 weeks gestation, 25% have morphological malformations.

The perinatal period extends from 28 weeks gestation to 7 days postnatally. Surveys indicate that approximately 25–30% of all perinatal deaths are caused by lethal congenital malformations. Neonatal deaths, up to 28 days postnatally, have a similar incidence (Young 1992^[4]).

Deaths from congenital malformations extend beyond the perinatal and neonatal periods into childhood making a significant contribution to childhood mortality. Population studies showed that in England and Wales in the period 1980–85, 27% of all deaths of infants under 1 year of age, 19% of all deaths of children aged 1–9 years and 7.5% of deaths of children aged 10–14 years were caused by congenital malformations.

From these figures it is apparent that at least 25–30% of human conceptions are malformed to some degree and that, generally, about 2–3% of all babies have at least one major malformation apparent at birth. The true incidence would also include malformations presenting later in life and would nearly double the value. Minor malformations of no medical or cosmetic significance are found in about 10% of the general population (Young 1992^[4]). For a summary of the incidence of malformations see **Table 3.3** .

It is beyond the scope of this text to give an in depth account of the thousands of defects now described. A selection of genome defects referred to in other parts of the embryology section will be outlined. The interested reader is recommended to study the list of some 385 diagnosable

Mendelian disorders listed by Connor (1992^[4]).

Chromosomal Defects

The defective separation of chromosomes at meiosis can result in tripling of individual chromosomes, *trisomy*. Apart from chromosome 1, each type of autosomal trisomy has been seen. Trisomy 16 is especially frequent in conceptuses but rarely survives to late gestation. Fetuses with a triploid or tetraploid chromosome complement, in the main, abort before term. Sex chromosome trisomies rarely abort, whereas sex chromosome deletions (XO) often abort. **Table 3.4** ^[5] shows the main chromosomal anomalies.

Unbalanced Deletions or Duplications

Chromosomal defects are also seen in cases where a portion of one chromosome is translocated to another location. If the resulting conceptus receives the full complement of chromosomes the condition is said to be a *balanced translocation* and no defect will result. If on the other hand the conceptus receives either a gain or loss of chromosomal material the condition is said to be an *unbalanced translocation* and the conceptus will either abort or develop multiple dysmorphic features. A number of conditions resulting from an unbalanced chromosomal complement have been described; an example is the *Prader-Willi syndrome* caused by microdeletion at 15q11–13. Affected individuals have hypotonia and poor swallowing in the neonatal period; this improves with age and overeating and obesity develop. The external genitalia are hypoplastic.

Single Gene Defects

Single gene defects are inherited as autosomal dominant, autosomal recessive, X-linked dominant or X-linked recessive traits. A number of such gene defects are shown in **Table 3.5** ^[6] with the mode of inheritance and the chromosomal location of the gene where this is known.

X-Linked Gene Defects

Vitamin D resistant rickets is an example of an X-linked dominant trait. Both males and females may be affected. In some other X-linked dominant conditions the affected males spontaneously abort.

X-linked recessive traits are more common; up to 368 have so far been identified and include:


- Duchenne muscular dystrophy (Xp21.2)
- Androgen insensitivity syndrome: testicular feminization, female phenotype, normal breast development, primary amenorrhoea, blind vaginal pouch, intra-abdominal testes
- Colour blindness: 3 Loci, blue (Ch7) and red and green (Xq28)

- Haemophilia: type A deficiency of factor VIII (Xq28); type B deficiency of factor IX (Xq27).

Multifactorial Disorders

Multifactorial disorders account for the vast majority of anomalies detected in the fetal and neonatal period. In some cases some of the genes involved have been identified and correlated to specific developmental events.

DiGeorge's syndrome has a microdeletion at 22q11 in 15-20% of patients. The condition demonstrates a failure of neural crest migration particularly into pharyngeal arches 3 and 4. Clinical features include absence (or near absence) of the thymus, parathyroids and thyroid, hypoplasia of the wall of the arteries derived from the aortic arches, outflow track malformations, dysmorphic faces, fish-like mouth, down-slanting palpebral fissures. The condition can be induced experimentally in *Hox a-c* nul mice.

Hirschsprung's disease is inherited as a multifactorial trait. One in 5000–8000 neonates is affected, more females than males. It results from failure of the neural crest neurons to invade the gut wall leading to an aganglionic section (see p. 235 )

Potter's sequence ensues from the absence of the kidneys and results in oligohydramnios, pulmonary hypoplasia, low set ears, squashed facies, talipes, amnion nodosum.

Wilm's tumour is nephroblastoma, diagnosed for 50% of cases within the first 3 years. It may be associated with microdeletions at 11p13. Patients also have aniridia, genitourinary malformations and mental and growth retardation. Wilm's tumour is often seen in patients with *Beckwith–Wiedemann syndrome* where a paternal deletion has been found at 11p15. Clinical features include macroglossia, anterior abdominal wall defects, high birth weight and hemihypertrophy.

Other congenital anomalies of the various body systems are considered to be of multifactorial origin; they are considered within the development of each system.

Mechanical Effects

A number of congenital malformations are caused by mechanical effects, particularly the reduction of amniotic fluid and formation of amniotic bands. Oligohydramnios will cause pulmonary hypoplasia and positional defects such as talipes. Other types of deformation include congenital dislocation of the hip, congenital postural scoliosis, pterygia and mandibular asymmetry. The development of amniotic bands, strands of amnion produced by premature amniotic rupture, can cause limb amputations and, by adherence of such bands to other parts of the fetus, a variety of other defects including facial clefts.

Twinning

Twinning occurs once in about every 80 births. Twins may be dizygotic (binovular or fraternal) or monozygotic (uniovular or identical), of which the former occurs more frequently.

Dizygotic Twinning

Dizygotic twins result from multiple ovulations which can be induced by gonadotrophins or drugs such as clomiphene (commonly used in patients with infertility). There is increasing evidence that the trigger for spontaneous multiple ovulations is higher follicle-stimulating hormone (FSH) levels in twin-bearing mothers. Studies on the Nigerian Yoruba tribe, which has a high incidence of dizygotic twinning, has shown elevated levels of FSH and luteinizing hormone (LH) compared to Caucasians, whereas in Japanese women, with a very low twinning rate, the FSH and LH levels were significantly lower (Nylander 1973^[4]; Soma et al 1975^[5]). Studies of hormone levels during the first four days of menstruation in women who had at least one set of twins, compared to those who had no twins, showed that FSH and oestradiol levels were both elevated in twin-bearing mothers (Martin et al 1984^[6]). Benirschke (1992^[7]) suggests that a gene may be responsible for higher FSH levels and thus be the cause of familial twinning. He notes that it is not yet clear whether the effect of increased FSH production seen in twin-bearing mothers is the result of a greater number of pituitary cells, more GNRH production or greater sensitivity of ovarian follicles.

Monozygotic Twinning

Monozygotic twins arise from a single ovum fertilized by a single sperm. At some stage up to the establishment of the axis of the embryonic area and the development of the primitive streak the formative material separates into two parts, each of which gives rise to a complete embryo. The resultant twins have the same genotype, but the description 'identical twins' is best avoided as most monozygotic twins have differences in phenotypes.

Almost all studies on the incidence of monozygotic twinning have shown that it occurs with the same frequency over ages and populations (Benirschke 1992^[7]). No causative factor has been identified. Although experimentally the separation of blastomeres in early gestation can result in two or more individual embryos, only in the nine-banded armadillo do such divisions occur regularly. In this species division of the early conceptus results in monozygotic quadruplets. Division of the later stages of conception may occur; the process of hatching of the blastocyst from the zona pellucida may result in constriction of the emerging cells and separation into two discrete entities. Interestingly, identical twinning occurs more frequently after human in vitro fertilization (9 sets of identical twins amongst 600 IVF births). The most likely explanation for this is damage to the zona pellucida, resulting in abnormal hatching of the human embryo through the narrow artificial gaps and subsequent inner cell mass splitting (Alikani et al 1994^[8]). There is a gradual decrease in the average thickness of the zona pellucida with increasing maternal age, which may be causally related to the increase in frequency of monozygotic

twinning with increased maternal age (Bulmer 1970^[4]; Alikani et al 1994^[4]).

It is thought that the ability of the early conceptus to regulate cell numbers and overall size is limited to the first 14 days of development. The precise time at which developmental regulation to produce twins ceases is unknown but is thought to correspond to this time span. After twinning monozygotic embryos enter a period of intense catch-up growth. Despite starting out at half the size, each twin embryo or fetus is of comparable size to a singleton fetus in the second trimester of pregnancy, but declines in relative size in the last 10 weeks of pregnancy.

Late separation of twins from a single conceptus may result in conjoined twins; these may be equal as in some varieties of 'Siamese twins', or unequal as in acardia.

Placentae in Twinning

The range of separation of the embryos is reflected in the separation of the extraembryonic membranes. The following types of placentation can occur (3.196^[4]):

- diamnionic, dichorionic separated
- diamnionic, dichorionic fused
- diamnionic, monochorionic
- monoamnionic, monochorionic.

The stage at which monozygotic twinning occurs will reflect the type of placentation seen. Monozygotic twinning may be diagnosed when monochorionic placentae are found, but only two-thirds of monozygotic twins have such placentae (with monoamnionic, monochorionic occurring in approximately 1–2% of twins). Of the dichorionic placentae, about 8% have been shown to be associated with monozygotic twins (Cameron 1968^[4]).

Monoamnionic, monochorionic placentae are associated with the highest perinatal mortality (>50%), caused both by entangling of the umbilical cords impeding the blood supply and by various vascular shunts between the placentae which may divert blood from one fetus to the other. Artery–artery anastomoses are the commonest followed by artery–vein anastomoses. If the shunting of blood across the placentae from one twin to the other is balanced by more than one vascular connection, development may proceed unimpaired. However, if this is not the case one twin may receive blood from the other leading to cardiac enlargement, increased urination and hydramnios in the recipient, and anaemia, oligohydramnios and atrophy in the donor.

Dizygotic twins have either completely separate chorionic sacs or sacs which have fused. Such placentae are separated by four membranes, two amnia and two choria; in addition such placentae have a ridge of firmer tissue at the base of the dividing membranes caused by the abutting of two expanding placental tissues against each other (Benirschke 1992^[4]). There may be size differences between diamnionic, dichorionic placentae because of intrauterine

competition for space.

Sex Ratio of Twins

The sex of twins will be the same for monozygotic twins (which have the same genotype) and may be different for dizygotic twins. Interestingly there is an excess of like-sex pairs among dizygotic twins (James 1971^[4]). Monoamnionic, monochorionic, monozygotic twins are most likely to be female, as are acardiac twins. A prospective twin study in Belgium indicated that the proportion of males was reduced in monozygotic twins, irrespective of chorionic status, and there was a marked reduction of male monoamnionic, monochorionic twins compared to a random occurrence (Derom et al 1988^[4]). The male/female ratio for all monozygotic twins was 0.487, while for monoamnionic, monochorionic twins it was 0.231; dizygotic twins had a ratio of 0.518.

Multiple Births

Multiple births greater than twinning, such as triplets or quadruplets, can arise from multiple ovulations, a single ovum, or both. It is most likely to be seen in women treated with drugs to stimulate ovulation.

For an excellent review of the types, frequency, inheritance and embryology of twinning, the diagnosis of their zygosity, the course and outcome of twin pregnancies, their possible evolutionary significance and some postnatal characteristics of twins, the interested reader should consult Bulmer (1970^[4]).

Prenatal Diagnosis and Treatment

In the last 20 years a variety of techniques has been developed for the prenatal detection and diagnosis of many congenital disorders. They have entered into obstetric practice and are an important part of antenatal care. There are two broad types of testing for congenital disorders: screening and diagnostic.

Prenatal screening may be undertaken using biochemical, genetic or ultrasonic methods. Such methods will detect a subgroup of those tested who are at higher risk of having a disease or disorder than the original population screened. *Diagnostic tests* are usually more complex and their aim is to give a definitive answer, particularly if a suspicion of a problem has been raised by a screening test. They may be non-invasive, like a detailed high resolution ultrasound scan, but many are invasive providing samples of different fetal tissue for analysis.

Biochemical Screening

Maternal blood samples may be taken at 16–18 weeks of pregnancy to screen for Down's

syndrome and open neural tube defects. There are four main markers of Down's syndrome: advanced maternal age, raised maternal serum human chorionic gonadotrophin (hCG), low maternal serum unconjugated oestriol (uE_3), and low maternal serum alphafetoprotein (AFP). Of these the most discriminatory serum marker for this condition is hCG. Examination of maternal blood samples will detect 60% of Down's syndrome.

Of the open neural tube defects, the most severe, craniorachischisis and anencephaly, are most easily detected by ultrasound. AFP leaks into the amniotic fluid and then into the maternal circulation in cases of open spina bifida. At 16–18 weeks levels of AFP in amniotic fluid are four times greater in open spina bifida than in unaffected pregnancies and there is a corresponding rise in AFP detected in the maternal serum. Such testing can detect 90% of cases, although the risk of false positives must be taken into account.

Genetic Screening

As carriers of recessive disorders are not diseased, genetic screening affects future generations rather than the individuals tested. Diseases that are currently included in screening programmes are mainly inherited as autosomal recessive disorders. The genes responsible for such disorders are very common in the general population, especially in some subgroups; for example, up to 17% of Cypriots carry thalassaemia, and 23% of West Africans carry sickle cell disease. The chances of affected individuals producing an affected offspring is therefore higher in these groups.


Options open to carriers of an inherited disease are: to remain childless; to select a partner who is not a carrier of the same disease; to use artificial insemination by donor or another form of assisted reproduction; to ensure that only a non-affected embryo implants by preimplantation diagnosis on an eight-cell embryo; to terminate a pregnancy found by antenatal diagnosis to be affected (Chapple 1992^[4]).


To assist with such decisions early information about the newly formed conceptus is desirable. New techniques, especially of molecular cytogenetics, will be at the forefront in the future to provide such information. At present accurate diagnosis of genetic defects relies on the collection of fetal cells from amniocentesis, the culture of these cells and the analysis of metaphase chromosomes resulting in the production of a karyotype. This may take 16–20 days. The use of DNA probes now permits the visualization of specific portions of chromatin in interphase. Detection of fetal sex, trisomy 18 and 13 can now be accomplished on examination of amniotic cells. It is suggested that the identification of a number of genetic defects will be possible via early chorionic examination in the future (Ferguson-Smith 1992^[5]).

Ultrasound Screening

Ultrasonography is an imaging technique used for screening which can detect structural abnormalities and malformations. It can be performed and repeated without risk to mother or fetus and most women in the United Kingdom have such a scan at 18 to 20 weeks of pregnancy.


Advanced ultrasonography requires high level skills and experience in the operator as well as knowledge of embryonic and fetal dysmorphology. A systematic anatomical survey of the head, face, brain, spinal cord, heart, chest, abdomen and its contents, urinary tract, skeleton and limbs is carried out.

One of the more common abnormalities that may be seen is spina bifida (3.197 ). The neural tube fails to close leaving a defect in the vertebrae, through which may protrude the meninges with or without the spinal cord (myelomeningocele and meningocele respectively). Often this lesion is detected by chance, or attention is drawn to it because associated cranial abnormalities may be seen, for example enlargement of the cerebral ventricles, or a lemon-shaped head due to concavity of the frontal bones. It may also present with an abnormal screening test, i.e. a raised maternal serum AFP. Spina bifida causes very severe handicap and most women with an affected fetus decide to terminate the pregnancy. The widespread use of screening has led to a 90% reduction in the incidence of neural tube defects at birth. Ultrasonography may detect up to 70% of all major malformations.

Ultrasound is also essential for the safe performance of all the invasive procedures. Such procedures are not without risk but in expert hands the fetal loss rate is increased by no more than 1–2%. The ultrasound scan reveals the intrauterine anatomy and enables the guidance of the instrument (needle or biopsy forceps) into the target, i.e. the amniotic cavity for amniocentesis, the placenta for chorionic villus sampling, the umbilical vein at the placental insertion of the umbilical cord or the intrahepatic tract of the umbilical vein for fetal blood sampling and the appropriate organ or structure for other tissues (3.198 .

Prenatal Treatment

A few of the abnormalities revealed by screening may be treated prenatally. Metabolic diseases and deficiencies have been treated, for example, by the administration of maternal corticosteroids for the prevention of respiratory distress in the premature infant, and by the administration of dexamethasone for congenital adrenal hyperplasia. Fetal cardiac arrhythmias can be treated by maternally administered agents.

One of the first fetal therapies, attributed to Liley (1963 ), was the intraperitoneal transfusion of blood for the treatment of severe erythrocyte alloimmunization. Access to the fetal circulation is now achieved by percutaneous umbilical blood sampling and intravascular transfusion using ultrasound guidance.

Fetal surgical techniques have developed recently, leading to a number of successful procedures which can promote normal growth during the latter part of pregnancy. Severe hydrothorax due to fluid in the pleural cavities causes pulmonary hypoplasia because pressure on the lungs and compression of the heart leads to heart failure and oedema (hydrops fetalis). These potentially fatal consequences can be prevented by placing a coiled catheter across the thoracic wall so that the hydrothorax can drain into the amniotic fluid and relieve the pressure in the chest. Urinary tract obstruction has been treated in utero, also hydrocephalus and, recently, congenital

diaphragmatic herniae. At present many of these operations have limited success and are only appropriate for selected cases. For a review of fetal therapy, particularly the experiences of the Fetal Treatment Programme at the University of California, see Kuller and Golbus (1992⁴).

Most malformations that are detected prenatally are definitively treated after birth. Often, however, awareness of the problem before birth leads to improved management and allows the parents to prepare themselves psychologically. In gastroschisis, for example, most of the gut has herniated through a small hole in the abdominal wall and floats freely in the amniotic fluid (3.199⁵). Towards the end of the pregnancy the condition of the gut can deteriorate and the aim is to deliver the baby before this happens. Careful ultrasonographic monitoring is required to time this correctly so that the baby and its gut are in optimal condition for immediate postnatal surgery. In such circumstances the results are excellent.

With the rapidly increasing possibilities for prenatal diagnosis and treatment and the greater understanding provided by the basic sciences, the fetus is now regarded as a patient and is the focus of the new discipline of fetal medicine.